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VASCULAR PATTERNS AND PERFUSION OF MUCOGINGIVAL TISSUES AND THEIR RELATION TO PERIODONTAL FLAP DESIGN

Kathleen Anne Lindell, M.S.

The University of Texas Graduate School of Biomedical Sciences at San Antonio

Supervising Professor: Donald G. Moskowicz, Colonel USAF

This investigation sought to describe the three dimensional microvascular anatomy of the periodontium utilizing silicone Microfil and acrylic perfusion techniques. A second aim of this study was to evaluate vascular response to surgical injury utilizing fluorescein angiography and laser doppler velocimetry. A total of seven Rhesus monkeys were used in this study. Four Rhesus monkeys were sacrificed followed by carotid artery cannulation, exsanguination, rinsing, and vascular perfusion of the head utilizing either Batson's #17 corrosion casting medium or silicone Microfil. Batson's perfused specimens (2) were then prepared for scanning electron microscopy and the Microfil specimens (2) were subjected to both

hematoxylin and eosin and cleared section analysis. In order to evaluate vascular response to surgical injury, three monkeys were studied with fluorescein angiography and laser doppler velocimetry following three different periodontal flap designs (narrow pedicle, wide pedicle, and envelope flap). Recordings were made at three time intervals (pre-surgery, post flap reflection and replacement, and following flap suturing), and at three tatooed tissue locations (coronal flap margin, base of flap, and midway between the previous two measurements).

Results: Silicone and acrylic perfusion techniques demonstrated unique, three dimensional features of the periodontal vasculature. Vertical feeder vessels were located primarily in thicker periodontal tissues associated with interradicular areas to include furcations. In keratinized tissue the greatest preponderance of vessels were orientated in a horizontal configuration and were often terminal branches of the vertical feeder vessels. The size, number and orientation of vessels was related to tissue interface, state of inflammation, and tissue thickness. Thin tissue demonstrated a more or less single layer of vertical and horizontal vessels whereas thick tissue demonstrated a tiered arrangement of vessels. Surprisingly, the vasculature of the periodontal ligament was centrally positioned and not located nearer bone.

Following surgical flaps, fluorescein angiography and laser doppler velocimetry demonstrated a greater decrease in blood perfusion in thin versus thick tissue. The proximity of vertical relaxing incisions (i.e., narrow versus wide pedicle flaps) was related to degree of perfusion change with narrow flaps most severely affected. In general, narrow thin flaps were most vulnerable to necrosis. Sutures placed with minimal tension did not adversely affect blood perfusion of surgically replaced flaps.

Following suturing and initial clot formation, vascular perfusion improved. The coronal flap margins always demonstrated less perfusion than the base of the flaps which routinely demonstrated an enhanced flow rate.

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Conclusions: The findings in this study suggest that the location of vertical incisions at the junction of thick and thin tissues (i.e., at tooth line angles) is associated with the best post surgical perfusion. Tissue thickness appears to be just as important to flap survival as the width to length ratio of flaps. Thesis (KT).

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Kathleen Anne Lindell

APPROVED:

Supervising Professor

26 May 1987
Date

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VASCULAR PATTERNS AND PERFUSION OF MUCOGINGIVAL TISSUES AND THEIR RELATION TO PERIODONTAL FLAP DESIGN

A

THESIS

Presented to the Faculty of

The University of Texas Graduate School of Biomedical Sciences

at San Antonio

in Partial Fulfillment

of the Requirements

for the Degree of

MASTER OF SCIENCE

By

Kathleen Anne Lindell, B.S., D.D.S.

San Antonio, Texas

May, 1987

DEDICATION

To my entire family, who have influenced so many decisions over the years; another step achieved.

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Conclusions: The findings in this study suggest that the location of vertical incisions at the junction of thick and thin tissues (i.e., at tooth line angles) is associated with the best post surgical perfusion. Tissue thickness appears to be just as important to flap survival as the width to length ratio of flaps.

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I. INTRODUCTION

Mucogingival flaps are routinely used in periodontics for access to underlying tissues or to correct soft tissue defects. Over the years, many flap designs have been proposed. To understand the biologic rationale for successful flap designs, a thorough understanding of the vascular supply to the mucogingival tissues must be obtained.

The vasculature of the periodontium has already been evaluated with a variety of techniques however, most of these techniques could only be accomplished in animal models because of their invasiveness.

The vasculature of animal models does not always resemble that of humans. Insufficient information has been available concerning the three dimensional arrangement and composition of the vasculature associated with keratinized and non-keratinized alveolar mucosa. Such information could be valuable in establishing a biologic rationale for tissue healing and as a scientific basis for new innovations in flap designs.

This study was intended to increase our understanding of the mucogingival vasculature and to correlate clinically successful flap designs with unique vascular distributions according to flap thickness, location, and vulnerability to surgically induced compromises in perfusion.

II. LITERATURE REVIEW

A. Anatomic Perfusion Techniques

Many techniques have been used to evaluate the vascular structures of the periodontium. Histologic sections using stains such as Hematoxylin and Eosin (H&E) have been often used during evaluation of healing.

Many of the vascular techniques have included the injection of some type of material into the vessels to help delineate them. Some of the earlier studies used gelatin mixtures that contained particles of carbon (Egelberg, 1966) or carmine (Cohen, 1959, 1960). India ink was injected intravascularly (Keller et al., 1955, Cohen, 1959, Castelli, 1965) as recently as 1984 (Caffesse et al.) for the purpose of evaluating healing flaps and appeared to work well with cleared or sectioned tissues. Cohen (1959) evaluated perfusions of India ink, carmine gelatin and a radiopaque material (bismuth oxychloride) and found that the carmine gelatin produced the best results.

Boyer et al.(1962) described a radiopaque technique using a precipitate of lead chromate in the vessels to enable evaluation on radiographs. However, while the major vessels could easily be identified using this technique, the smaller vessels and spatial relationships between vessels were difficult to evaluate.

Other materials such as latex (Kindlova, 1965, Birn, 1966) and silicone materials such as Microfil (Nuki and Hock, 1975, Bassingwaighte et al., 1974, and Cutright and Hunsuck 1970) have also been injected intravascularly. In these studies (following injection) the tissues were cleared using various methods to change the optical properties of the soft and hard tissues to differentially allow the passage of light. By using

these techniques, the spatial relationship of the vessels could be observed, and this technique permitted the further sectioning and staining of tissue. Additionally it ensured the distention of the vessels so they could be more easily identified.

Microfil in a radioactive form was also used by Brady and Cutright (1971) to indirectly quantify the amount of blood present in the mandible of the rat.

Vital microscopy has been used to facilitate visualization and description of vascular units in the gingival margin (Wayland and Hock, 1974, Nuki and Hock, 1975) as well as those associated with the sulcus (Hansson et al., 1968). The limitations associated with this technique seem obvious, in that the tissue surface cannot be violated in order to evaluate an undisturbed vasculature, thus limiting the technique to visualization of only the most superficial vessels.

Adenosinetriphosphatase activity (ATPase) in vascular endothelium has also been utilized to identify vascular relationships in periodontal tissues (Carranza et al., 1966).

The technique employed in this study used vascular corrosion casts made with a type of methylmethacrylate (Batsons No. 17 Corrosion Casting Compound). Prior to a healing study of gingiva (Nobuto et al., 1987), corrosion casts had been used in the study of many organ systems but never used to evaluate the periodontal vasculature. The technique requires the injection of catalyzed methylmethacrylate into vessels and then allowing the injection mass to polymerize and harden. The soft tissues were then corroded from the surface of the infused plastic leaving a cast of the vascular tree. Like most of the previous materials, the substance is able to infuse the capillary beds, allowing them to be reproduced accurately.

Several commercially available casting media can be obtained, and the most frequently used and best documented include Mercox (Japan Vilene Co. Ltd. 7-20, Nihonbashi 3-chrome, Chuo-ku, Tokyo 103, Japan), methylmethacrylate (Fluka A. G., Chemische Fabrik, Ch-9470 Bucho SG, Switzerland) and modified Batson No. 17 (Polysciences, Inc. Warrington, Pa.). Polymerization "set" times are very similar with 5 minutes or less working time allowed from the addition of the catalyst and accelerator. However, the shrinkage of the material tends to differ (the most distortion occurring with the methylmethacrylate (20% by volume), and least in the modified Batson No 17 (1% by volume) leaving Mercox as the intermediate (6%) (Lametschwandter et al., 1984)).

B. Anatomy of the Oral Vasculature

Utilizing the previously listed techniques, several authors have extensively described the vascular supply to the periodontium.

The vascular supply to the facial soft tissues in the posterior maxillary and mandibular regions has been shown to be similar in humans and Rhesus monkeys, with these zones being supplied by the posterior superior alveolar arteries in the maxilla and branches of the facial arteries in the mandible. Since neither the mental foramen nor mental arteries exist in the Rhesus monkey, the periodontal tissues of the mandibular incisors are supplied by terminal branches of the sublingual arteries (which penetrate the hard tissues in the symphysis region). Finally, the maxillary anterior teeth are supplied by the ascending terminal branches of the greater palatine artery and the facial soft tissues of this region are supplied by branches of the facial artery (Castelli 1965).

The blood supply to the periodontal ligament (PDL) increases from the anterior to the posterior teeth, with the supply consistently greatest in the gingival one third of the ligament. The least number of vessels are found in the middle third of the root in anterior and bicuspid teeth, but the middle and apical thirds are approximately equal in molar teeth (Birn 1966). A plexus of vessels exists in the periodontal ligament with the larger vessels parallel to the long axis of the tooth. A significant amount of vascular branching is evident with some branches feeding into the plexus from adjacent bone. This plexus is located nearer to the osseous surface of the PDL than to the tooth (Carranza et al., 1966).

The flat capillary network (which is characteristic of the periodontal ligament) ends at the margin of the alveolar bone while other vessels of the periodontal ligament continue to course toward the marginal tissues to anastomose with supraperiosteal vessels.

The vasculature of the dentogingival junction in dogs was evaluated in both a state of health and disease (Egelberg, 1966). Healthy sulcular tissue demonstrated no vascular loops, except in the free marginal tissue areas. A network of vessels was always seen underlying the surface of the crevicular epithelium with vessel diameters ranging in size from 7-40 microns. This arrangement of vessels, which was intimately related to the epithelial cuff (Waerhaug, 1952), was called the crevicular plexus. Using vital microscopy of sulcular tissues in dogs, Hansson et al (1968) described three zones in healthy tissue. The marginal zone was 60-70 microns wide and contained no recognizable vessels. The middle zone ranged from 90 to 100 microns, ran parallel to the epithelium and was characterized by a plexus of vessels that ranged from 7-10 microns in diameter. In most areas of the middle zone, small vessels (15-20 microns) existed in loop formations that

extended toward the avascular layer. The apical zone contained vertically oriented vessels (up to 40 microns in diameter) which tended to run coronally to the bottom of the gingival crevice (in a direction perpendicular to the vessels of the middle zone).

Utilizing information gathered from vital microscopy and Microfil perfusion, Nuki and Hock (1975) described the oral aspect of the free gingiva in dogs and cats with no discernible difference noted between maxillary and mandibular canine and molar regions. Vessels in the crestal gingiva essentially represented anastomoses between the crevicular and buccal networks of vessels. Arterioles greater than 25–44 microns in diameter tended to run parallel to the gingival margin and successively branched into (1) smaller arterioles, (2) precapillary arterioles (15–18 microns wide), and (3) capillaries underlying the epithelium of the crevice and buccal gingiva. Capillary units consisting of a minimum of two terminal arterial capillaries, four primary venular capillaries, and a variable number of connecting vessels were also described.

Chronically inflamed areas of sulcular tissue demonstrated an increase in the vascular content with the narrow and straight vessels associated with health being replaced by loop formations. Hematoxylin and eosin preparations have vividly demonstrated loop formations associated with epithelial projections into the connective tissue (Egelberg 1966). Hansson et al (1968) demonstrated an avascular zone in the inflamed tissues, but it was narrower than that found in health. The other distinguishable zone consisted of wide tortuous capillaries and venules forming loops perpendicular to the surface of the tissue. When inflammation increased, both the winding nature of the vessels and their width increased. This finding has been reconfirmed by Nuki and Hock in the oral aspect of the

gingival margin, where they showed that as inflammation increased, vessel width increased by 5-10 microns, length increased by 400-1000 microns, and the elongated vessels became "twisted, spiraled or looped".

Kindlova (1965) described glomerulus-like capillary loops along the epithelial attachment. The capillary network under healthy oral epithelium formed very short capillary loops; however in the presence of inflammation, the loops formed new branches and anastomoses and developed dilations and varicosities which leaked the latex material, (suggesting disturbances in the integrity of the vessel wall). As inflammation increased, the greatest changes in capillaries occurred in the vessels supplying the epithelial attachment.

Using microspheres to evaluate the quantity of blood flow to the tissues of the gingiva, Folke and Stallard (1967) found that areas of inflammation demonstrated an increased number of spheres in the tissues, suggesting there was an increase in the vasculature per unit volume of tissue. This finding agreed with the previously described histologic and perfusion studies which demonstrated increases in the length and width of vessels.

The same authors noted that the marginal and attached gingival tissues contained capillary loops that ran perpendicular to the outer surface and received their arterial supply from periosteal vessels. Kindlova (1965) noted that the major vessels supplying the mucogingival tissues coursed vertically along the periosteum and gave off branches to more superficial layers. This palisading effect had been described as early as 1881 by Wedl. Cutright (1970) described the major vessels coursing anteriorly and coronally, (superficial to the periosteum) which branched to supply the mucosa, gingiva, and alveolar process. Cutright (1970) also described the

vessels of the periosteum as a dense reticulated vascular plexus which completely enveloped the bone and arose from nearby connective tissue vessels that branched as they penetrated periosteum.

In general, previous studies have shown that the vasculature in the mucogingival tissues of the periodontium course more or less vertically along the periosteum with branches supplying the more superficial tissues. In addition, the periodontal ligament, alveolar process, and mucogingival vasculature tend to frequently anastomose along their interfaces.

C. Physiologic Perfusion Techniques

Historically, there have been many methods utilized to evaluate blood perfusion in soft tissues including: vital microscopy, high speed cinematography, microspheres, radiolabeled microspheres, radioisotope clearance, hydrogen clearance, plethysmography, temperature clearance, dermofluorometry and most recently laser doppler velocimetry. Many of these were reviewed by Bishop and Dorman (1968).

Vital microscopy has allowed an in vivo observation of superficial capillary flow, but deeper structures are not recorded. In addition, this technique requires the use of expensive and cumbersome optical equipment, but since it is non-invasive, it can be readily utilized in human subjects. Using vital microscopy, two authors (Staple 1955, Forsslund 1959) have reported "sludging" within vessels in areas of gingivitis. In similar techniques, high speed cinematography was utilized by Hock and Nuki (1975) to trace individual red blood cells as they coursed past superficial vascular landmarks.

Microspheres have been used to evaluate perfusion on several occasions by infusing them into a major artery and using the relative number of

microspheres per unit volume of blood as an indicator of blood flow. Quantitative data could be obtained by radiolabeling the microspheres and evaluating the radioactivity in the tissues. Limitations in the use of microspheres include permitting a single measurement of flow and restricted use of this radioactive material on laboratory animals.

Another use of radiolabeled material includes the injection of radioisotopes into tissues followed by determination of the clearance rate by monitoring the radioactivity remaining in the tissue over time. This technique is also limited to animal use and has suffered from difficulties in data analysis. Hydrogen clearance has been used in a similar manner to evaluate blood flow to a region, but again similar problems occur in analysis.

Dermofluorometry (or fluorescein microfluorometry) gives a quantitative assessment of blood flow to a site based on the fluorescent emission. To this date a dermofluorometer has not been used intraorally but the use of fluorescein perfusion in conjunction with photography has been reported (Mormann and Ciancio, 1977). Using injections of fluorescein dye, oral soft tissue incisions and flaps have been evaluated for perfusion in much the same way skin flaps have been evaluated in the plastic surgery literature.

Dyes have also been utilized to predict flap viability. Patterson and Chir (1968) utilized disulfine blue injections (instead of fluorescein) to visualize perfusion of differently designed skin flaps. The dye techniques have demonstrated a relatively accurate representation of the quality of tissue perfusion but local differences or continuous changes over time cannot be accurately evaluated.

Other approaches to evaluating the quality of vascular perfusion have used an indirect measure of blood flow in the tissues. Venous occlusion

plethysmography has been considered the most reliable method for measuring skin blood flow. A similar technique using electrical impedance plethysmography has been utilized intraorally to measure pulp and oral soft tissue blood flow (Neidle and Goldberg 1978). The latter procedure depends on the relatively low electrical impedance of a volume of blood when compared to hard tissue. Impedance plethysmography is applicable to human subjects, but one disadvantage to this technique is that the electrode must touch the tissue to be monitored, and contact pressures may alter the capillary flow in the region.

Another indirect method of measuring blood flow is temperature clearance. A heat diffusion transducer, heated by a known current, is placed into the sulcus and the voltage across the transducer is constantly monitored. Diffusion of heat from the device is altered by the change of blood flow through the adjacent tissues; however, the reliability of the device is questioned because heat produced by the transducer may in turn alter the flow to the area.

Laser doppler velocimetry which is a continuous and non-invasive technique for evaluation of blood flow to gingival tissues has been developed over the last decade. This technique depends on the doppler shift of coherent (laser) light reflected from the tissue. The frequency shift of light is due to movement of particulate blood cells within the tissue relative to the unshifted signal ("background noise") received from stationary tissue, and is therefore directly related to blood flow parameters. This technique offers several advantages over previously discussed methods of evaluating vascular perfusion: 1) it is non-invasive, and does not require tissue contact, 2) it can be used on any accessible surface, 3) the signal is continuous thus allowing for changes over time,

and 4) it is specific to superficial tissue surfaces, the exact depth of laser penetration appears to vary with different manufacturers from 0.5 mm to 3.0 mm deep (Johnson, Taylor et al 1984, Kviety et al., 1985, Baab et al., 1986).

D. Evaluation of Blood Flow

The laser doppler method for blood perfusion evaluation has been compared to several other techniques for linearity, reproducibility, and intersubject variability, as well as predictive value in assessing flap survival.

Smits et al. (1986) demonstrated an almost perfect correlation (r=0.99) of the laser methodology with a known velocity on a calibration wheel. Utilizing the kidney tissue of rats, they also demonstrated a high correlation with electromagnetic flowmeter (EMF) generated readings (r=0.91) and microsphere analysis (r=0.91). Although a slightly lower correlation was found in gracilis muscle r=0.74 and r=0.78 respectively, a high correlation with EMF was demonstrated by Kiel et al. (1985). Kviety et al. (1985) (following laser doppler, hydrogen clearance, and microsphere estimations of perfusion in feline jejunum) demonstrated a high correlation of laser doppler and microsphere analysis (r=0.82). Microsphere data correlated well with EMF as a standard (r=0.96) but hydrogen clearance tended to overestimate total flow as it tended to selectively measure highly perfused mucosa. Larrabee et al (1983), Liu et al (1986), Marks et al (1984) and Cummings et al (1984) individually noted that the predictive capability of the laser doppler instrument for flap survival (when measured at the surgical appointment) underestimated the actual survival at two weeks, but the values recorded at 24 hours or later accurately predicted survival.

Fluorescein dye evaluation of perfusion in the same flaps demonstrated equal or slightly better early predictive values but was similar to the laser at 24 hours. In these studies, excellent perfusion was considered to be >100mV or full fluorescence, marginally effective perfusion ranged between 40mV and 100mV or incomplete fluorescence, and a poor prognosis was predicted when values of <40mV or no fluorescence were obtained. Comparing several techniques of blood flow monitoring, Kviety et al. (1985) estimated that on the Periflux PF 1c laser doppler flow meter (Perimed KB, Stockholm) 1 volt is approximately equal to 10 ml flow / min / 100gm tissue.

The laser doppler has found a place not only in research but also in the clinical setting since it has been used in gastrointestinal tissue, skin flaps, nasal mucosa, retina, and intraoral tissue. The sites selected for measurement with the doppler can be more discrete than any comparable instrument or technique because of the narrow separation existing between the transmitting and receiving fiberoptic bundles.

A laser doppler flowmeter has recently been used to study blood flow in oral tissues. DeRijk et al. (1980), in a preliminary study on one human subject, reported "a direct readout of the average flow and of the cardiac pulsations in flow" in gingival tissues. In another study, Baab et al. (1986), using a Periflux PF1d (Perimed KB, Stockholm, Sweden) instrument, evaluated blood flow to the interdental papillae, free and attached gingiva, and alveolar mucosa to assess the effects of heat, cold, pressure and occlusal forces on regional blood flow. Their results revealed that flow patterns differed depending on the tissue type, but were consistent within each type. Mucosa consistently had higher mean flow rates than free or attached gingiva: both heat and cold produced initial hyperemia followed by a return to baseline; pressure caused ischemia followed by a reactive

hyperemia in all tissues, and occlusal forces induced a localized marginal ischemia. The reliability of repeated measurements was high (0.75 $p<0.001$).

E. Application to Soft Tissue Flaps

The use of P^{32} (Ohmori and Kurata, 1960), vital dyes (Patterson and Chir, 1968), and fluorescein angiography (Mormann and Ciancio, 1977) has allowed a more scientific application of the knowledge of vascular anatomy as it relates to clinical (surgical) practice. Ohmori and Kurata, using rabbits and various types of skin flaps, evaluated perfusion utilizing injections of radioactive phosphorus immediately post surgery and several days later. Their observations indicated that the intrinsic circulation of simple pedicle flaps is maintained when the ratio of width and length is 1:2; beyond that length the skin flap had insufficient circulation to survive. Patterson and Chir evaluated skin flaps on the flank of the pig. Pedicle flaps consistently demonstrated excellent perfusion at the base of the flap with diminishing dye saturation as the distance from the base increased. They reported that the dye penetration advanced two thirds of the flap length within 30 seconds. The dye penetrated the remaining one third of the flap over a 10 to 30 minute interval. Following this extended time period the dye color became more intense but further extension of the perfused area was not noted. A line of survival demarcation developed at five to six hours post surgery with no alteration seen over the next seven days. The tissue beyond the line of demarcation converted to a dry eschar by the seventh post surgical day. The extent of flap survival depended heavily upon the blood supply to the pedicle. Inclusion of segmental vessels to the pedicle flap provided the best results, with decreased

survival length evident with the ligation of essential vessels. There also appeared to be a greater reduction in surviving length following ligation of the arteries as compared to division of veins. If at least one complete vascular bundle was not included in the pedicle, the surviving length was usually reduced by approximately one-third.

Mormann and Ciancio (1977), in a human study, evaluated the 24 hour effect on vascular perfusion resulting from various flap and incision designs. A full thickness horizontal incision interrupted the blood flow coronally, thereby reinforcing the concept of apical to coronal direction of blood flow, while internally beveled incisions at the gingival margin did not interrupt the blood flow to the marginal area. Having more than one source of vascular supply to the crestal margin (periodontal ligament and mucogingival) resulted in minimal interruption of blood flow as determined by fluorescein angiography. Vertical incisions without elevation of a full thickness flap demonstrated a disturbance to the blood flow, but when full thickness mucoperiosteal flaps were elevated, a substantial reduction (50%) in flow to the area was demonstrated at 24 hours. Tall narrow flaps demonstrated a more critical disturbance in blood flow than shorter or wider flaps and the placement of significant tension on the flap following suturing was sufficient to induce focal necrosis.

III. STATEMENT OF THE PROBLEM

In order to evaluate and understand the healing responses of the periodontium, a thorough understanding of the relationships of the blood vessels to each other and to related tissues is critical. In previous studies, the vasculature of the oral and perioral structures were described in animals and humans but only a few in vivo studies have been accomplished in humans due to inherent risks or practical limitations imposed by the techniques. The anatomical rationale for the design of periodontal flaps has not been adequately explained in previous studies, nor has an adequate explanation been given as to why the same design will heal well in one region and necrose in another. Other questions arise concerning use of split thickness grafts and pedicle grafts of excessive length. Answers to these questions may be related to the quantity and arrangement of the vascular supply to the periodontium. Part I of the following study was designed to clarify the present knowledge of the periodontal vasculature. This study was innovative by supplementing standard H&E and cleared histologic sections with vascular corrosion casts, a technique not previously utilized for studying the periodontal vasculature. With this technique, a third dimension of the vascular organization can be compared to the more frequently used two dimensional histologic techniques. Part II of the study was designed to evaluate how variability in flap dimensions will affect blood flow perfusion as determined by laser doppler velocimetry and fluorescein angiography. Information gathered from both portions of this study should help clarify the biologic basis for healing and repair on the basis of the adequacy of post surgical flap perfusion.

IV. MATERIALS AND METHODS

A. Vascular Anatomy

PART I of this study verifies the vascular anatomy of the facial mucogingival tissues distal to the canine teeth in the maxillary and mandibular arches of the Rhesus monkey.

Three techniques for visualization of perfused vasculature were accomplished: 1) H&E stained histologic sections, 2) cleared sections and 3) vascular casts. Silicone Microfil (Canton Bio-Medical Products, Inc. Boulder, Colorado) perfusion was accomplished in animals used for both H&E stained histology and cleared specimens. Perfusions of Batson's #17 anatomical corrosion compound (Polysciences, Inc. Warrington, Pennsylvania) were utilized to produce vascular casts.

1. Hematoxylin and Eosin Stained Histology and Cleared Sections

Two animals were anesthetized with Ketamine (100 mg/ml), 5 mg/kg body weight, and Rompum (100 mg/ml) 0.1 ml intramuscularly. Endotracheal tubes were placed and the animals were ventilated with room air. In each animal, a midline incision was made from near the mandibular symphysis to the suprasternal notch. Blunt dissection to expose the common carotid arteries bilaterally was accomplished. Two size 0 silk ties were placed around each artery. At this time the animal received 5000 units of heparin IV. This was allowed to circulate for 3–4 minutes before an overdose of pentobarbital was administered to the animal. The midline incision was continued to allow access to the chest and abdominal cavities; the animal was then eviscerated. With access from the chest cavity via removal of the

medial aspect of each clavicle, the common carotid arteries were cannulated with 14 gauge angiocatheters.

The animal was secured to the table using rope ties. The table was then tilted approximately 60 degrees trendelenburg and a towel roll placed under the shoulders of the animal, thus allowing the level of the cannulas in the carotid arteries to be superior to the head. Using a pump to supply a continuous infusion pressure and utilizing tubing with a Y connector, the carotid arteries were simultaneously rinsed with heparinized saline to thoroughly evacuate the heparinized blood from the vasculature of the head. Two liters of fluid containing warm saline, 35-40 mg adenosine deabinase (a vasodilator), and an additional 5000 units of heparin were pumped into the vessels until the return fluid remained clear. Two or more liters of 10% buffered formalin immediately followed the saline flush and were infused until the soft perioral tissues developed noticeable stiffness. An attempt was made to maintain a continuous flow of fluid through the tubing so that pressures within the vessels would remain nearly constant to maintain vascular dilatation during fixation. Recall that formalin tends to constrict blood vessels as it fixes the tissue. The pump and tubing used for irrigation and fixation were disconnected at this time, leaving the angiocatheters in place in the common carotid arteries.

Approximately 190 cc of Microfil was then mixed, following the manufacturers directions (100 ml dilutent: 80 ml compound: 9 ml catalyst). Using 12 cc syringes, the Microfil was injected into the carotid arteries via the cannulas. Both sides were filled simultaneously to insure adequate perfusion. The animal was maintained in the trendelenburg position until the Microfil began to set

(approximately 15-20 minutes). The monkey was placed in a cold room overnight to allow time for the final set of the Microfil.

At necropsy, the maxilla and mandible were excised then divided at the midline into right and left sides. The specimens were slowly decalcified over a three month period using sodium citrate and formic acid (20%) with constant magnetic stirring.

The right maxillary and mandibular portion of each animal were sectioned and stained with hematoxylin and eosin. Maxillae were sectioned through the long axis of the teeth, separating palatal from facial aspects. Maxillae (facial and palatal) and mandibles were divided into blocks for further sectioning (Appendix A). The blocks of tissue were processed for paraffin embedding and sectioned approximately 7 microns thick in either the frontal or horizontal plane. Hematoxylin and eosin staining followed the outlined steps from the AFIP guide. All sections were reviewed but only representative sections were photographed (using Ektachrome 64 slide film and Nikon microphot light microscope).

The left maxilla and mandible of each animal were left intact until the clearing process was completed. The process used for clearing followed the protocol recommended by the manufacturer. Increasing concentrations of alcohol (25%, 50%, 75%, 90%, 95%, absolute), changed twice daily, (allowing two days at each concentration) dehydrated the tissues. Following dehydration, the specimens were submerged in methylsalicylate for the clearing process. Each maxilla and mandible was divided into three large blocks of tissue that were later subdivided into sections in either the horizontal or frontal plane (Appendix B). Each section was 2-3 mm in thickness.

Photographs were taken of the large blocks prior to sectioning and photographs were made of all sections using a Nikon SMZ-10 stereomicroscope and reflected light.

2. Vascular Casts

Two animals were anesthetized with Ketamine, 5 mg/kg body weight, and Rompum 0.1 ml (100 mg/ml) intramuscularly. Oral endotracheal tubes were placed and the animals were ventilated with room air. Incision and dissection were accomplished in the same manner as with previous animals. Five thousand units of heparin were administered intravenously three to five minutes prior to euthanasia which was accomplished with an overdose of pentobarbital. Fourteen gauge angiocatheters were inserted and tied to place in the common carotid arteries with size 0 silk ligatures. Trendelenburg positioning of the animal was accomplished until the entire head was below the level of the cannulas (approximately 60 degrees). Using the pump and tubing as described previously, warm saline containing heparin and adenosine deabinase was used to flush the vessels of the head and neck. The tubing was then disconnected, leaving angiocatheters in place.

Approximately 200 cc of Batson's #17 (methylmethacrylate) material mixed (in two batches) according to the manufacturers instructions (200 ml base solution; 24 drops promoter and 24 ml catalyst), with the minimum recommended quantity of catalyst being used to allow for maximum working time. The material was immediately injected into the carotid cannulas with 12 cc syringes. Both sides were perfused simultaneously. The monkey was allowed to remain in trendelenburg position until the material began to set (approximately 5-10 minutes).

The animal was placed in a cold room for overnight storage to allow for final polymerization of the methylmethacrylate.

The monkey was decapitated and excess soft tissues were removed from the head. The remaining cranial and facial structures were suspended in a large glass beaker containing 3 molar potassium hydroxide (KOH) for the first 4 - 6 hours, then 1 molar KOH thereafter. The beaker was placed on a magnetic stirring table under a fume hood. The specimen was rinsed in water and the solution was changed periodically over a 3 - 5 day interval until all apparent soft tissues had been corroded from the surface of the vascular casts, leaving hard structures (bone) intact for support and reference.

Individual sections from vascular cast (Appendix C) were carefully separated and lifted from the bony surface and placed in individual beakers for further corrosion (with concentrated KOH solution) for approximately one hour. Preparation of the specimens for critical point drying was accomplished using increasing concentrations of alcohol followed by increasing concentrations of freon. The second animal was processed differently by the addition of an ultrasonication step (for 10 minutes in a glass cleaning solution - Micro lab cleaner, International Products Corp, Trenton NJ)) added prior to vascular cast dehydration with alcohols and freon. The addition of the ultrasonication step to specimen preparation resulted in cleaner specimens when viewed with the scanning electron microscope. All specimens were critical point dried in an Autosamdri - 814 (Tousimis Research Corp, Rockville, Md) then placed on specimen mounts and sputter coated with gold and palladium using a Hummer VI (Anatech Ltd, Alexandria Va). A Phillips SEM 515 was used for all scanning electron

microscopy. Black and white photographs of each specimen were taken with a Polaroid camera attachment and Polaroid T-55 film.

B. Laser Doppler Velocimetry

PART II of the study involved the evaluation of changes in perfusion of mucogingival tissues following periodontal flap reflection. The instrument used to evaluate vital perfusion of the periodontal flaps was the prototype instrument for TSI, a laser doppler flowmeter (Laserflo blood perfusion monitor; TSI, Inc, St. Paul, Mn).

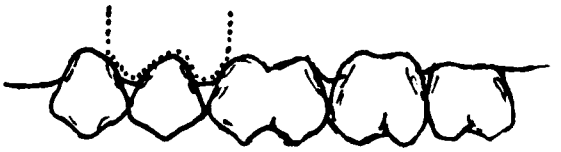
1. Flap Design

Three different buccal flap designs were evaluated for the maxilla and mandible: (1) 20 mm wide envelope flaps (with no vertical releasing incisions). (2) pedicle flaps with a base:height ratio of 3:1 (12 mm wide and 4 mm long apical-coronally). (3) pedicle flaps with a base:height ratio of 1:3 (4 mm wide and 12 mm long apical-coronally) (see figure 1). All full thickness flaps in this study had internally beveled incisions, and were carefully returned to their original locations.

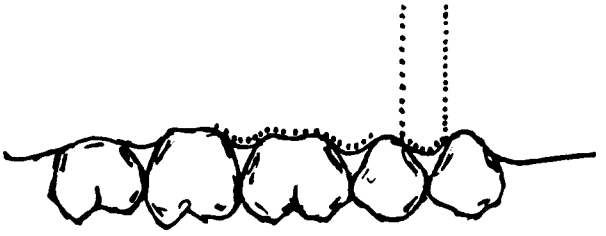
2. Presurgical Preparation

Two weeks prior to surgical manipulation of tissues, reference tatoos of india ink were placed with a 26 gauge needle tip in the buccal gingiva and mucosal tissues of 3 animals (figure 2). The right side of each animal was tatooed to establish the 3:1 wide pedicle flap designs. The left maxilla and mandible of each animal was tatooed for the 1:3 narrow pedicle and envelope flap design. Existing calculus was

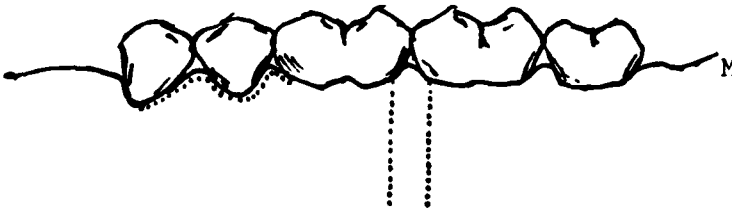
Figure 1 FLAP DESIGNS FOR LASER DOPPLER EVALUATION



Maxillary Left



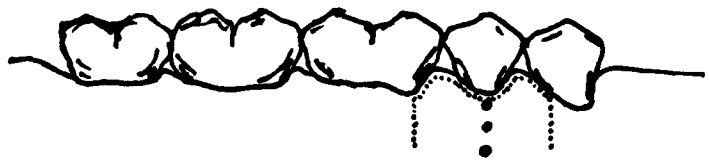
Maxillary Right

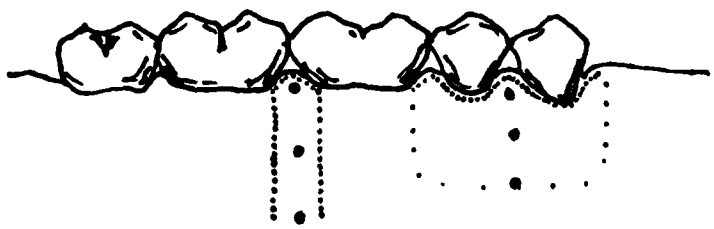


Mandibular Left

Mandibular Right

Figure 2 LOCATION OF DOPPLER READINGS (TATOOS)





removed from the supra and subgingival tooth environment with a curette.

3. Surgical Procedures

Three animals were used for this portion of the study. Two flap procedures of each design were accomplished on each animal. The animals were preoperatively sedated with intramuscular Ketamine (50 - 60 mg depending on weight), Rompum (10 mg, 0.1 cc) and Atropine (40 ug, 0.1 cc). Oral endotracheal intubation was accomplished and the animals were placed on a 2 liter flow of oxygen, 20 - 40 cc of halothane and 2 liters of nitrous oxide. An open intravenous line was maintained with 5% dextrose in water.

The head of the monkey was secured in a position that allowed the probe tip of the doppler instrument to be perpendicular to the measured surface. Towel rolls were used to stabilize the head and surgical tape was employed to secure the cheek retractor. Doppler measurements were made after placing the probe tip (3 mm diameter) in light contact with the tissue then gently elevating and lowering the tip to just clear the tissue surface (by means of a micromanipulator attached to a non-mobile framework). Two readings were usually made adjacent to each tatoo. A third reading was sometimes necessary if there was a wide variation in the previous values. In all instances the two highest reproducible readings from each site were recorded. Site recordings at each tatoo were made at three vertical locations: coronally near the margin, at the base of the flap, and midway between the previous two locations. Flap management consisted of careful elevation and immediate replacement of the flap to its original position. The flap was

subsequently held in place with gentle pressure for 3-5 minutes, or until bleeding stopped.

Both pre and post suturing recordings were annotated for the three locations, margin, midway and base of the flap. Two to four sutures (5-0 Dexon) were usually adequate to secure the flap in place prior to final doppler measurements.

Following all recordings and prior to extubation of the animals, for each surgical day, one surgical site in each animal was photographed with Ektachrome 64 slide film following intravenous injection of sodium fluorescein (0.6 -0.7 cc, depending on weight). Serial photographs (with five second lapse intervals) were obtained, using a Wood's lamp (ultraviolet light) for illumination. Photographs were continued until no further color change could be detected.

On day one of surgery, all 1:3, narrow pedicle flap procedures were performed on all animals. On day 4, the opposing side of each arch was similarly surgerized utilizing the 3:1, wide pedicle flaps. Finally, on day 6, envelope flaps were created adjacent to the healing narrow pedicle flaps (see figure 1).

C. Data Analysis

Data analysis was performed utilizing analysis of variance to evaluate perfusion values and to note significant trends or findings.

D. Animal Care

All animals were used and cared for in compliance with DOD directive 3216.1 "The Use of Animals in DOD Programs", AFR 169-2 "Animals in Department of Defense Research and Training", and NIH publication #78-23 "Guide for the Care and Use of Laboratory Animals".

V. RESULTS

A. Normal Anatomy

Maxillary and mandibular vascular tissues of four Rhesus monkeys were prepared for microscopic examination utilizing three techniques of preparation: 1) H&E stained sections, 2) cleared sections, and 3) vascular corrosion casts. Total sections included: 100 H&E sections, 72 cleared sections and 90 corroded specimens. These were viewed (respectively) by light, dissecting, or scanning electron microscopy. An attempt was made to include vertical and horizontal sections of both thick and thin regions in each type of preparation.

1. Periodontal Ligament

The vessels of the periodontal ligament form an anastomotic network which surrounds the root of the tooth. The major blood supply appears to course in an apical-coronal direction with many vascular anastomoses. Most of the vessels appear to enter the PDL area through the apical portion of the ligament space (Plate 1), while other connecting vessels enter at various levels along the lateral surface of the cribiform plate (Plate 2). In H&E sections, some of the vessels appear to be located adjacent to tooth and bone, however, the majority were positioned midway between these calcified surfaces (Plates 1,3,4) (figure 3).

2. Periosteum

A very dense globular layer of corrosion casting material was found adjacent to the surface of the bone with vessels occasionally

Plate 1

A. PERIODONTAL LIGAMENT VESSELS IN CORONAL H&E STAINED SECTION AT MANDIBULAR MOLAR FURCATION ENTRANCE (original magnification 10x). Network of periodontal ligament vessels course coronally (a) and anastomose with supraperiosteal vessels of the gingiva (b) (vessels containing Microfil).

B. PERIODONTAL LIGAMENT VESSELS IN CLEARED SECTION OF MAXILLARY MOLAR (original magnification 1x). Periodontal ligament vessels (a) surrounding buccal root coursing vertically from apical origin. Large supraperiosteal vessels (b) apical to mucogingival junction in cross section, smaller vessels coursing vertically (c) in gingiva and into papilla.

Plate 2

A. CLEARED SECTION OF MANDIBULAR PREMOLAR WITH VASCULAR CONNECTIONS FROM PERIODONTAL LIGAMENT THROUGH BONE TO MUCOGINGIVAL TISSUE (original magnification 1 x). Penetrating vessel connecting PDL and supraalveolar vessels through bone (arrow). A change in density of vascular loops of superficial tissues at mucogingival junction (mgj) is evident.

B. CLEARED HORIZONTAL SECTION OF MANDIBULAR MOLARS WITH BONE TO MUCOGINGIVAL TISSUE AND PERIODONTAL LIGAMENT VESSELS (original magnification 1.5x). Connecting vessels (facial and lingual) between supraperiosteal and osseous vessels (a). Vessels penetrating cribiform plate into PDL (b). Connections between adjacent periodontal ligaments (c). Change in density of superficial vessels from gingiva (g) to alveolar mucosa (m).

Plate 3

A. H&E STAINED HORIZONTAL SECTION OF PERIODONTAL LIGAMENT (original magnification 10x). Vessels in PDL (a) are located between bone and tooth (on average) at the midpoint of the calcified surfaces. Several large vessels (b) sectioned in longitudinal and cross section are seen in the overlying loose connective tissue.

B. CLEARED SECTION OF MAXILLARY MOLARS WITH VASCULAR CONNECTIONS BETWEEN PERIODONTAL LIGAMENTS (original magnification 1.5x). Vessels coursing between adjacent periodontal ligaments (a) and between supraperiosteal vessels and PDL (b). Note the dense arrangement of vessels along the gingival surface (c) with larger vessels near the bony surface.

Plate 4

A. H&E STAINED HORIZONTAL SECTION WITH VASCULAR CHANNELS CONNECTING BONE AND OVERLYING SOFT TISSUES (original magnification 10x) (arrow). Position of periodontal ligament vessels centrally located (a).

B. H&E STAINED HORIZONTAL SECTION WITH VASCULAR CHANNELS CONNECTING BONE AND PERIODONTAL LIGAMENT (original magnification 10x) (arrow). Note periodontal ligament vessel location and longitudinal sections of vessels in overlying connective tissue (a).

penetrating into the underlying cortical plate (Plates 5,6). This densely vascular layer appears to be an integral part of the vascular cast. Some histologic specimens also demonstrate endothelium lined vascular spaces in the cambium layer of the periosteum (Plate 7). Above the cambium layer is a much less vascular region which corresponds to the reticular zone of the periosteum (figure 3).

3. Free Gingival Margin (Sulcular Aspect)

The flat network of anastomotic vessels in the periodontal ligament continues along the connective tissue surface underlying the healthy epithelium of the sulcus until the gingival margin is reached. Blood vessels in the marginal region also change configuration from the flat network seen along the gingival sulcus and periodontal ligament to capillary loops of moderate length that tend to have a slightly dilated and coiled appearance when compared to vessels underlying the more distant oral epithelium (Plates 8, 9, 10). In health, the epithelium of the gingival margin at the entrance to the sulcus retains many of the characteristics of the oral epithelium in the region. H&E preparations reveal smooth stratified squamous epithelium lining the sulcus without epithelial proliferation.

H&E preparations in sulcular regions of moderate inflammation demonstrate extensions of the epithelium into the connective tissue in rete ridge-like formations with an associated mild infiltrate of inflammatory cells (Plate 11). Corrosion casts of this region demonstrate an increase in dilation and twisting of the capillaries with further apical extension along the sulcular aspect than seen in

Plate 5

A. SEM OF VASCULAR CORROSION CAST IN FACIAL TISSUE OF MAXILLARY FIRST PREMOLAR WITH PERIOSTEAL VESSELS (35.8x magnification, bar = 1mm). Vessel penetrations along the periosteal surface severed at entrance to bone (a). A dense layer of periosteal capillaries underlies the supraperiosteal vessels (b). View from along a vertical section demonstrates large horizontal and oblique vessels.

Plate 6

A. SEM OF CORROSION CAST FROM MAXILLARY FIRST PREMOLAR FACIAL TISSUES WITH A DENSE PLEXUS OF VESSELS ALONG THE BONY SURFACE (17.2x magnification). Vertical section through thin mucogingival tissues (gingival margin on right). Dense plexus of vessels adjacent to bone surface (arrow) in periosteum.

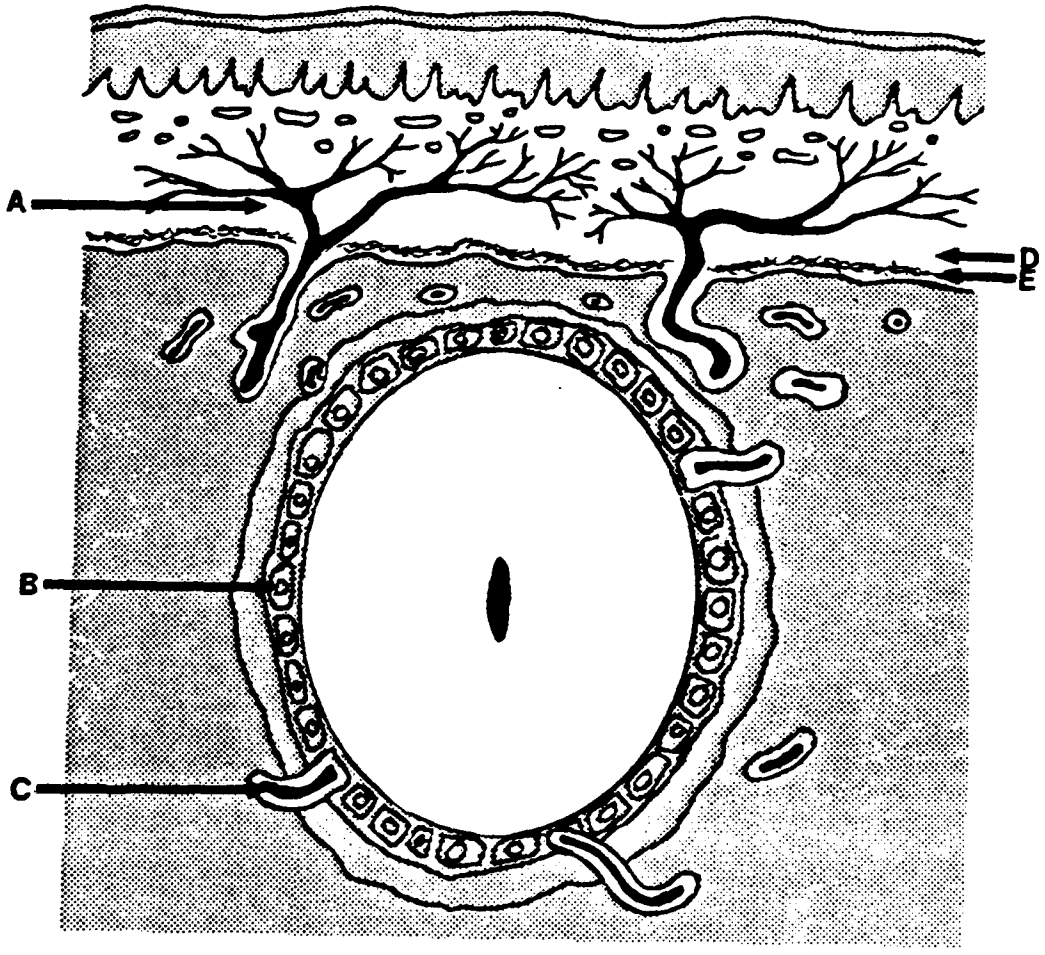
B. SEM (143x) SECTION OF SPECIMEN A ALONG PERIOSTEAL PLEXUS OF VESSELS. Penetrating vessels evident (a) approximately 15 um diameter. Mark = 0.1mm.

Plate 7

A. H&E STAINED SECTION OF GINGIVA WITH PERIOSTEAL VESSELS ALONG THE BONE SURFACE (arrow) (original magnification 50x).

B. SPECIMEN A (100x). Endothelial cells lining vascular spaces (arrow). Vessels lie between Sharpey's fibers inserting into the bony surface.

Figure 3 DIAGRAMATIC VIEW OF PERIODONTAL LIGAMENT AND ORAL SOFT TISSUE VASCULATURE



A penetrating vessel into overlying connective tissue

B centrally located vessels in PDL

C penetrating vessel from bone into PDL

D reticular zone of periosteum

E periosteal plexus of blood vessels

Plate 8

A. H&E STAINED SECTION OF HEALTHY GINGIVAL MARGIN TISSUES. (original magnification 10x). Thin layer of sulcular epithelium (a) develops ridge formations at the gingival margin (b). External epithelium demonstrate rete ridge formations with capillary loops in connective tissue papilla.

B. VASCULAR CORROSION CAST OF HEALTHY MARGINAL TISSUE. Vertical section from mandibular premolar region (13.8x magnification). Large horizontally coursing vessels are present in mucosal tissues (a), with some vertically oriented vessels (b) in the marginal tissues.

Plate 9

A. VASCULAR CORROSION CAST OF MARGINAL TISSUE (Plate 8) (34.4x magnification). The healthy sulcus (S) is lined by a network of vessels, many coursing vertically (a). Loop formations are present on the oral aspect (O) of the tissue (between connective tissue papillae) and at the entrance to the sulcus (b).

B. VASCULAR CORROSION CAST OF HEALTHY MARGINAL TISSUE (SULCULAR AND PERIOSTEAL SURFACE) (22.2x magnification). Major vessels course horizontally (a), with some vertically oriented vessels seen only in the papillae (b). The anastomosing plexus of vessels from the PDL (c) is approaching the marginal tissue, where loop formations begin.

Plate 10

CORROSION CAST OF SULCULAR ASPECT IN REGION OF MILD INFLAMMATION (28.8x magnification). Very dense region of capillaries is present near the gingival margin (a). Capillary loops of the free gingival margin (b) with a slender straight configuration are contrasted to the engorged, dilated, and twisted vessels of the inflamed sulcus (c).

Plate 11

A. H&E STAINED VERTICAL SECTION IN A REGION OF MODERATE SULCULAR INFLAMMATION (original magnification 10x). Mild inflammatory infiltrate is present along the sulcus, projections of epithelium (a) are located apical to the marginal tissue, and dilated capillaries (b) are present along the epithelium.

B. VASCULAR CORROSION CAST FROM MANDIBULAR PREMOLAR GINGIVA WITH MODERATE SULCULAR INFLAMMATION (26.4x magnification). Capillary loops along the sulcus (a) increase in number and appear dilated and more twisted than capillary loops on the oral surface. Note the extensive horizontal vasculature in the mucosal region (b) and the vertical feeder vessel in the papilla (c).

healthy specimens. No apparent changes are seen under the oral surface (Plate 11).

H&E sections of regions with severe inflammation are associated with a more extensive epithelial proliferation with rete ridge formation appearing along the entire sulcus. Oral surface epithelium may also demonstrate some acanthosis to the extent that the two epithelial surfaces contain only a small interface of marginal connective tissue. A large number of chronic inflammatory cells are present in the connective tissue near the base of the sulcus (Plate 12). Vasodilatation can be seen in the region of the connective tissue papillae between epithelial proliferations; and a thin layer of epithelium can be seen proliferating apically along the root surface. Corrosion casts of a region with severe sulcular inflammation demonstrate marked dilation and proliferation of the crevicular vessels resulting in "glomerular-like" groups of capillaries at the end of engorged capillary stalks located between ridges of proliferating epithelium (corroded from the specimens) (Plate 13).

4. Free Gingival Margin (Oral Aspect)

In health, the capillary loops projecting between the rete ridges become longer, narrower, and relatively more straight in configuration than sulcular capillaries (Plates 8, 9, 10, 11).

5. Surface Capillaries

The configuration of these vessels differs in the regions of the attached and free gingiva as compared to the alveolar mucosa (Plates 14, 15, 16). The most superficial blood vessels associated with the

Plate 12

H&E STAINED SECTION OF SEVERELY INFLAMED SULCULAR TISSUE (original magnification 10x). Acanthosis of sulcular epithelium is evident. Dilated vessels are present underlying the sulcular epithelial tissue (arrow).

Plate 13

A. CORROSION CAST OF SEVERELY INFLAMED MARGINAL TISSUE (19.5x magnification). Sulcus located on right (S). (Oral surface (O) damaged in processing).

B. ENLARGED VIEW OF SULCUS FROM A (97x magnification). "Glomerular-like" capillary projections with severely coiled vessels at the ends of dilated capillaries (arrows).

Plate 14

VASCULAR CORROSION CAST OF FACIAL INTERRADICULAR TISSUE FROM MAXILLARY FIRST AND SECOND MOLARS (10.2x magnification, Mark = 1 mm). Changes in surface capillary configuration evident at the mucogingival junction (arrows).

Plate 15

A. CORROSION CAST OF ATTACHED GINGIVAL TISSUE (specimen in Plate 14) (21.2x magnification) Mark = 1 mm. Long thin capillary loops along the free gingival margin (a) change to shorter loops in the remaining attached tissue (b). A sharp demarcation in loop form is seen at the mucogingival junction (mgj). Many of the underlying vessels course in a horizontal or oblique direction (c).

B. CLEARED SECTION FROM MANDIBULAR SECOND PREMOLAR (original magnification 1.5x). Attached gingival tissues demonstrate dense plexus of vessels along the surface (arrow) with larger vessels nearer the bone surface supplying the region (a).

Plate 16

A. VASCULAR CORROSION CAST OF MUCOSAL TISSUE SEEN IN Plate 14. (21.1x magnification). Capillary loops (a) beneath mucosal epithelium (corroded from specimen) are much shorter and somewhat twisted as compared to attached tissue (Plate 15). The majority of underlying vessels visible course horizontal or oblique (b).

B. CLEARED SECTION FROM APICAL TO MUCOGINGIVAL JUNCTION IN MANDIBULAR MOLAR REGION. (original magnification 1.3x). A thin layer of capillaries is present along the external surface (a), with a major network of larger vessels adjacent to the periosteal surface (b) giving off branches to more superficial tissues.

oral surface of the epithelium are of capillary size. The long thin hairpin-like loops of the free gingival margin convert to slightly shorter thin loops along the surface of the remaining keratinized tissue. At the mucogingival junction an abrupt change occurs in capillary configuration. The capillary loops of the alveolar mucosa surface are much shorter and are slightly twisted forming a flat network.

6. Interproximal

Numerous vessels can be observed exiting from the bony surface and coursing coronally to supply the overlying tissues. In the connective tissues adjacent to the epithelial surface the blood vessels tend to be small in diameter. Inflammation in the region is graphically demonstrated with the appearance of numerous inflammatory cells in the connective tissue adjacent to the epithelium, evidence of epithelial proliferation and, in some areas, ulceration of the surface. The vessels in this subepithelial region now tend to be much larger in diameter than those noted in the healthy specimen (Plate 17). In the interproximal col region, healthy tissues are characterized by a scant number of inflammatory cells and a uniform, thin layer of stratified squamous epithelium lining the surface with no distinctive epithelial proliferation into the underlying connective tissue (Plate 17).

7. Vascular Orientation Related to Tissue Thickness

The deeper connective tissue located between the sulcular and oral epithelium contains larger vessels that eventually branch to form the capillary loops located beneath both of the epithelial surfaces (oral

Plate 17

A. H&E STAINED TISSUE FROM HEALTHY INTERPROXIMAL COL (original magnification 10x). (Papillary region contains moderate-severe inflammation. Several vascular channels are evident (a) coursing from the bone. Small blood vessels are noted (b) underlying the mildly inflamed thin layer of epithelium.

B. H&E STAINED TISSUE FROM INFLAMED INTERPROXIMAL COL (original magnification 10x). Large numbers of inflammatory cells are evident with prominent epithelial proliferation into ridge formations (a). Dilated blood vessels are evident throughout the underlying tissue (arrow).

and sulcular). These vessels originate primarily from the supraperiosteal region with a smaller contribution from the periodontal ligament. The orientation of the major vessels varies depending on location and tissue thickness. It is evident from reviewing all specimens that interproximal regions tend to have thicker tissues than radicular areas. In thicker tissue, the larger vertically oriented arterioles give off branches directed horizontally to supply blood to the thinner radicular regions (figure 4). In thick gingival tissue, horizontal and oblique vessels appear more prevalent in the deepest and most superficial layers of the connective tissue, with vertically oriented feeder vessels featured in middle layers.

Thin tissues, having a smaller connective tissue core, tend to have a different arrangement of blood vessels. The interproximal and furcal regions of keratinized tissue contain most of the major apical-coronal feeder vessels, as can be seen in horizontal sections (Plates 18, 19, 20). In the papillary regions, branches of these vessels fan coronally and horizontally, dividing into smaller arterioles that eventually branch to form the capillary loops seen on the surface of the attached tissues and the free gingival margin. The larger horizontal vessels located over root prominences form a mat of densely packed arterioles with a thin superficial layer of capillaries forming the loop extensions corresponding to the connective tissue papillae under the rete ridges of epithelium (Plate 18).

The major vessels supplying the marginal areas of the flap become sandwiched between sulcular and oral capillaries. The palisading vessels in the keratinized gingiva overlying the surface of bone tend to have more anastomoses than those in the loose connective tissue

Figure 4 DIAGRAM OF VESSEL ORIENTATION IN KERATINIZED TISSUE

Interproximal regions and furcations contain most of the vertically oriented vessels supplying the keratinized gingiva.

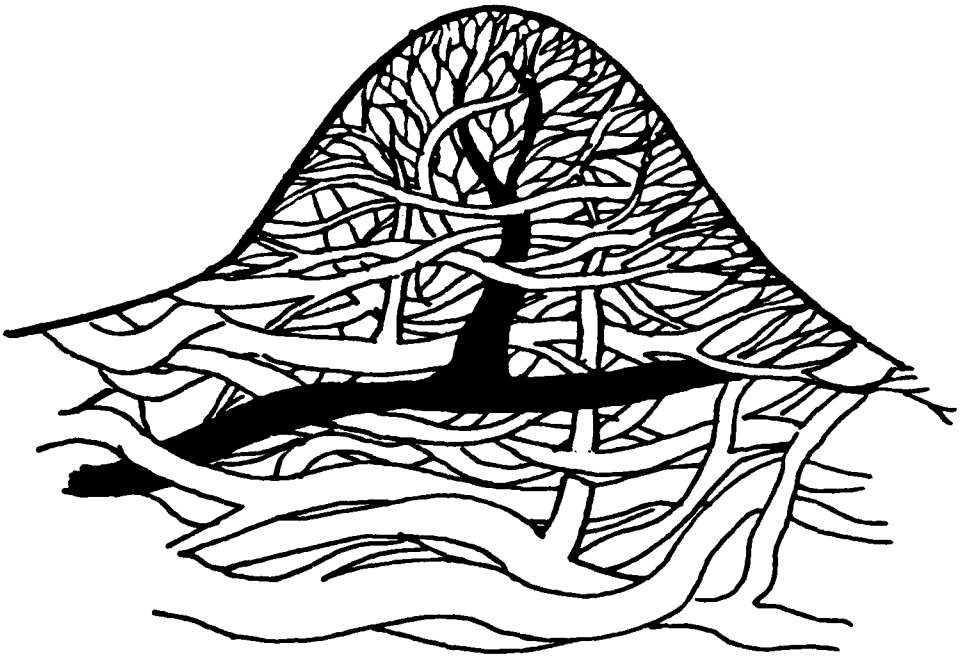


Plate 18

A. VASCULAR CORROSION CAST OF THIN MARGINAL TISSUE FROM A MAXILLARY PREMOLAR VIEWED FROM THE PERIOSTEAL AND SULCULAR SURFACE (24.2x magnification). Capillary loops of the gingival margin (a) blend with the network of vessels originating in the periodontal ligament (b). Various sizes of vessels are observed along the periosteal surface with multiple anastomoses.

B. VASCULAR CORROSION CAST OF SAME SPECIMEN AS 18A VIEWED FROM SECTIONED APICAL SURFACE (24.2x magnification). Note the large apical-coronal vessels in the region of the papilla (arrows), and horizontal plexus of vessels in the radicular region between papillae (hv).

Plate 19

A. H&E STAINED HORIZONTAL SECTION OF THIN GINGIVAL TISSUE WITH LARGEST VESSELS COURSING VERTICALLY IN THE INTERPROXIMAL REGION (a) (original magnification 10x). There is a slight increase in connective tissue thickness in the region of the vessels. Oblique and longitudinal sections of vessels are seen nearer the root prominence (b). Note the position of the vessels in the periodontal ligament, midway between calcified surfaces.

B. CLEARED HORIZONTAL SECTION FROM MAXILLARY CENTRAL AND LATERAL INCISORS DEMONSTRATING GROUPS OF LARGER VESSELS LOCATED IN INTERPROXIMAL REGIONS (arrow) WITH FEW LARGE VESSELS NOTED OVER ROOT PROMINENCES (original magnification 1x)

Plate 20

A. HORIZONTAL H&E STAINED SECTION NEAR THE LEVEL OF THE ALVEOLAR CREST WITH THE LARGEST VESSELS SEEN IN THE THICKEST AREA OF THE PAPILLARY TISSUE (arrows) (original magnification 10x).

B. CLEARED SECTION OF VERTICALLY ORIENTED VESSELS IN THE INTERPROXIMAL REGION (arrows) (original magnification 1x). Anastomoses of periodontal ligament vessels and those of the facial tissues and bone are demonstrated well (arrows).

found in the alveolar mucosa, resulting in less artificial separation during processing.

In keratinized gingiva, the area occupied by blood vessels tends to be somewhat thicker than similar regions at the mucogingival junction. In several specimens, the region of the mucogingival junction tended to be extremely thin, often the thinnest part of the specimen. Blood vessels in this region are of much smaller diameter than those found more apically in the mucosa and course parallel to the mucogingival line. Vertically oriented vessels occur in periodic sequence with interproximal papillae (Plates 18, 21) (figure 5).

In the alveolar mucosa, the connective tissues are much less dense and the associated blood vessels tend to be arranged in tiers (Plates 22, 23, 24, 25, 26, 27). Artificial separation of these dense vascular layers was seen following processing for SEM (as they easily separated into distinctive tiers with only a few interconnections). Most of the major blood vessels in the mucosa were oriented horizontally and obliquely (Plates 21, 22, 28). Vertical vessels were more sparse in comparison but still represented a greater density than those seen in keratinized tissue. At the vestibular fornix, larger vessels with less specific orientation are present. Even in these areas, the larger vessels tended to be in the deeper and central layers with smaller vessels predominating along the epithelial and periosteal surfaces (Plates 23, 24, 26) (figure 6, 7).

Major differences between thick and thin tissues occur in the alveolar mucosal tissues apical to the mucogingival junction. The layering of vessels in thick tissue is evident but to a much greater extent than in thin tissue. Instead of a single serpentine layer of

Plate 21

A. VASCULAR CORROSION CAST OF THIN TISSUE SECTIONED THROUGH AN INTERPROXIMAL PAPILLAE (26.4x magnification). Periosteal surface. Large horizontally coursing vessels in the mucosa (m) with a large vertically oriented feeder vessel (fv) into the papilla (p).

B. VASCULAR CORROSION CAST OF THIN TISSUE (REMAINING HALF OF PAPILLA FROM SECTION A) (26.4x magnification). Periosteal surface. Mucogingival junction (mgj) more easily identified with large horizontally oriented vessels in mucosa and capillary loops along free gingival margin (gm). Vessels found at the mucogingival junction are almost exclusively horizontal.

Figure 5 VERTICAL FEEDER VESSEL LOCATIONS

In thin tissues, vertical vessels are almost exclusively located at interproximal and furcal sites.

In thicker tissues, most vessels are interproximal and furcal, but some can be found over radicular surfaces.

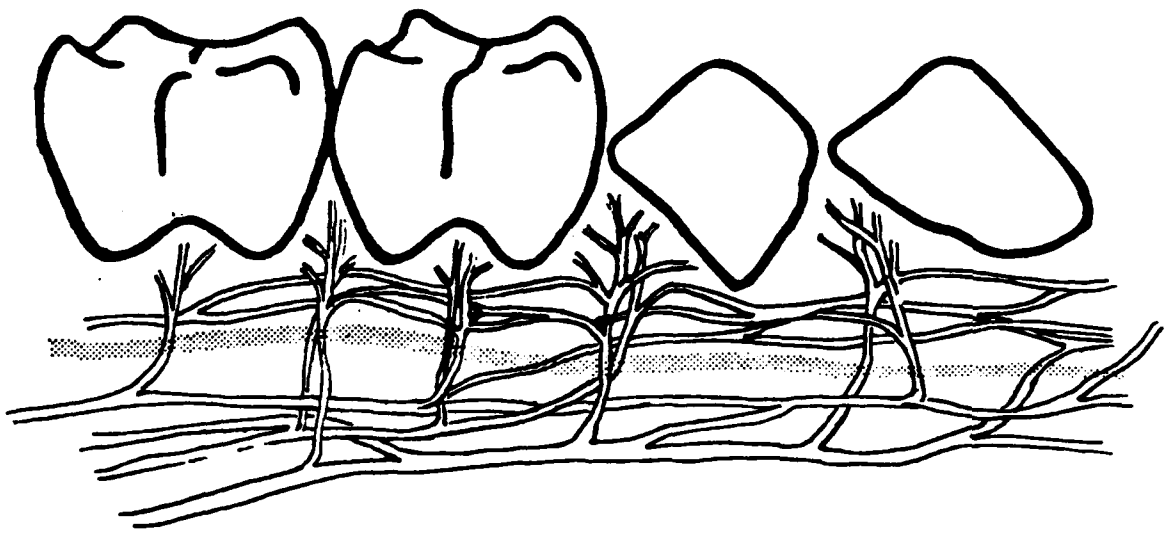


Plate 22

VASCULAR CORROSION CAST OF THIN MUCOGINGIVAL TISSUE ALONG A VERTICAL INCISION (27.6 x magnification). (top) The gingival margin contains small vessels oriented both horizontally (a) and vertically (b). Mucosal vessels (bottom and middle) lie in a single plane along the bone, nearly stacked vertically. Most of the larger vessels are cut in cross section and course horizontally (c). Near the vestibule tiers of vessels can be seen and are easily separated with preparation (d).

Plate 23

A. VASCULAR CORROSION CAST OF HORIZONTAL SECTION IN THIN TISSUE SECTIONED APICAL TO THE MUCOGINGIVAL JUNCTION (22.2x magnification) (Apical to specimen A plate 24). Thin layers of capillaries, arterioles, and veins are present in a horizontal orientation. Large feeder vessels (arrow) course vertically into the more coronal papilla.

B. VASCULAR CORROSION CAST OF HORIZONTAL SECTION IN THIN TISSUE SECTIONED NEAR THE VESTIBULE (22.2x magnification). Layers of vessels are present (arrows) with variable sizes. The largest vessels course vertically as feeder vessels (fv) into the papillary region.

Plate 24

A. VASCULAR CORROSION CAST (VERTICAL INCISION) OF THICK MUCOGINGIVAL TISSUE FROM A MAXILLARY SECOND MOLAR (19.5x magnification. Bar = 1 mm). Keratinized tissue of gingiva (folded at left) with epithelial surface down. Mucogingival junction (mgj) is the narrowest portion of the cast. Large vessels of the mucosal tissues are present in several layers with many (cut in cross section) coursing horizontally (arrows).

B. H&E STAINED SECTION OF THICK GINGIVAL TISSUE (original magnification 10x). The area of the mucogingival junction (mgj) has the least thickness. Large feeder vessels (fv) are seen coursing into the gingival margin.

Plate 25

A. VASCULAR CORROSION CAST OF A VERTICAL SECTION IN VERY THICK TISSUE FROM NEAR THE VESTIBULE OF THE MAXILLARY MOLARS (18.7x magnification). Largest vessels are near the periosteal surface (arrow). Specific orientation of vessels is not as prominent as in thinner or more coronal tissues. Many layers of vessels are seen in this specimen.

B. VASCULAR CORROSION CAST OF A VERTICAL SECTION THROUGH THICK TISSUES FROM MAXILLARY MOLARS (more coronal to specimen A). Tissue layers more evident (arrows) with larger vessels in deeper layers and primarily coursing in a horizontal direction. Capillary loops under the epithelial surface are evident (c)

Plate 26

A. VASCULAR CORROSION CAST OF A HORIZONTAL SECTION IN THICK MUCOSAL TISSUE SECTIONED NEAR THE MUCOGINGIVAL JUNCTION OF A MAXILLARY PREMOLAR (30.2x magnification Bar = 1 mm). Distinct layers of vessels are present (arrows) with capillary loops (c) along the epithelial surface. Larger vessels are in middle and deep layers. Vertically oriented feeder vessels (fv) are arranged in groups associated with the interradicular tissues.

B. CLEARED SPECIMEN OF MANDIBULAR MOLAR AND PREMOLAR REGION SECTIONED APICAL TO THE MUCOGINGIVAL JUNCTION (original magnification 1.3x). Layers of vessels within the soft tissues can be distinguished (arrows).

Plate 27

A. VASCULAR CORROSION CAST OF HORIZONTAL SECTION NEAR THE VESTIBULE IN THICK TISSUE OF A MAXILLARY PREMOLAR (30.2x magnification). Epithelial surface (top) contains fewer capillary loops than more coronal sections. Large vessels (some greater than 0.5 mm diameter) are present and oriented in a less organized fashion than more coronal vessels.

B. CLEARED SECTION OF MANDIBULAR MOLAR AND PREMOLAR REGION SECTIONED NEAR THE VESTIBULAR FORNIX (original magnification 1.3x). Vessels are seen in layers (arrows) with largest vessels near the bony surface and less organization in the vestibule (V)

Plate 28

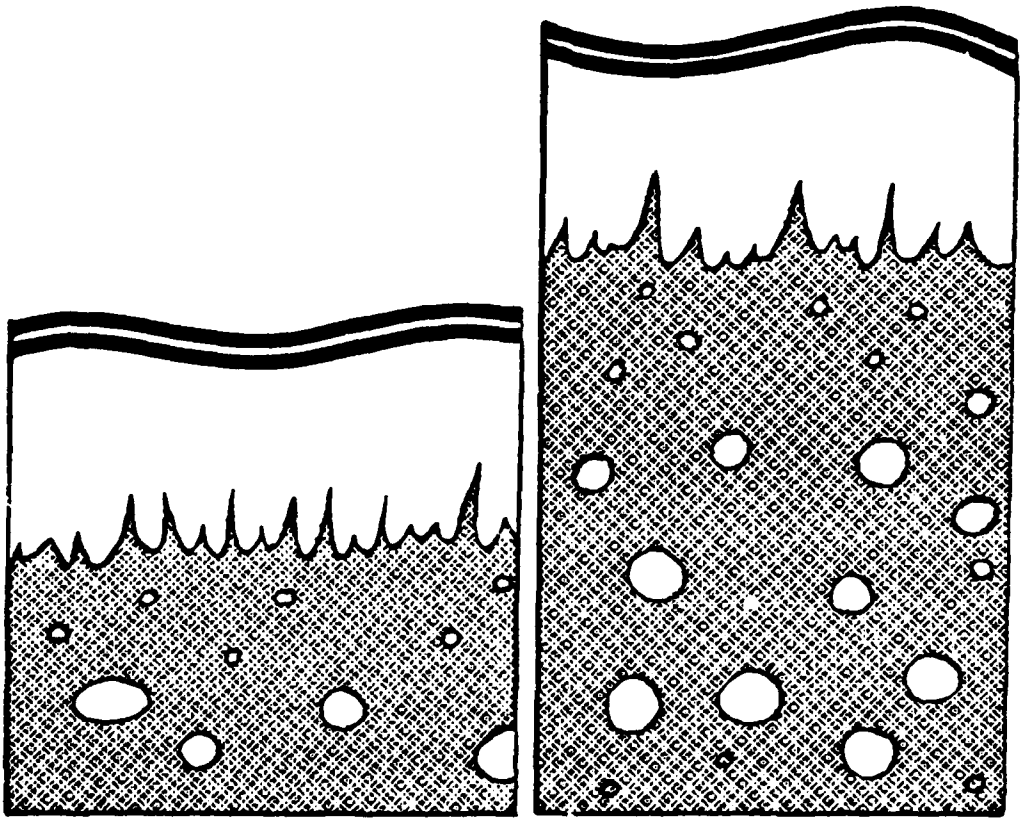
A. H&E STAINED SECTION OF MUCOGINGIVAL TISSUE NEAR FURCATION ENTRANCE WITH NUMEROUS HORIZONTALLY ORIENTED VESSELS IN MUCOSAL TISSUES (a) AND SMALLER VESSELS IN KERATINIZED TISSUE (b) (original magnification 10x).

B. H&E STAINED SECTION OF THE TORTUOUS COURSE TAKEN BY A LARGE FEEDER VESSEL (arrow) INTO THE GINGIVAL MARGIN (original magnification 10x).

Figure 6 PALISADING EFFECT OF VESSELS RELATED TO TISSUE THICKNESS

In thin tissues, larger vessels form a single layer in deeper regions of the connective tissue near the bony or root surface.

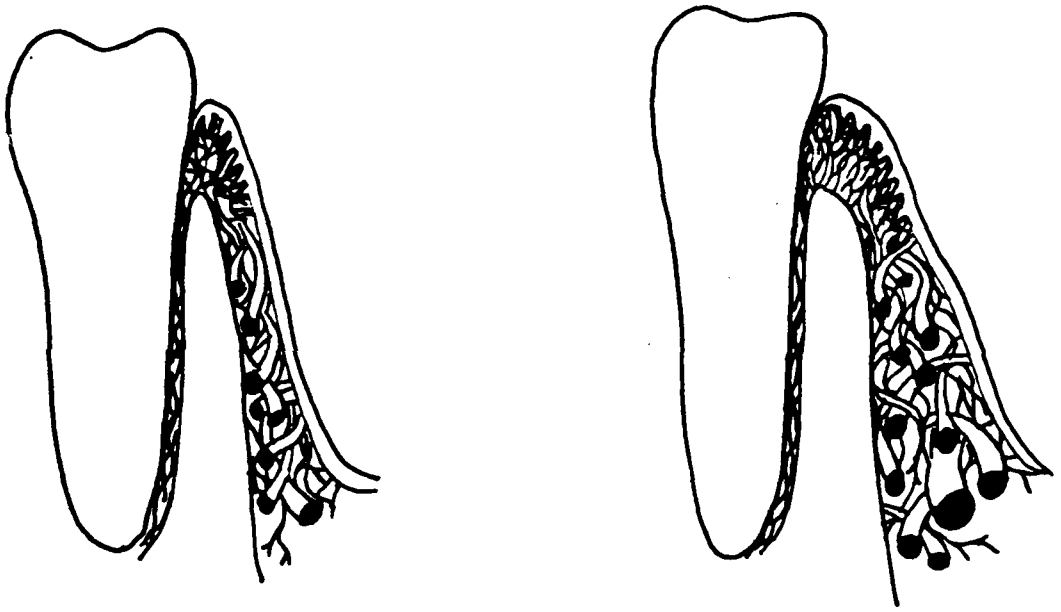
Thicker tissues demonstrate layers of larger vessels in deep and middle regions of connective tissue.



Thin Tissue

Thick Tissue

Figure 7 PALISADING EFFECT OF VESSELS RELATED TO MUCOSAL THICKNESS



major horizontal and oblique vessels seen in thin tissues, the larger vessels of thick tissue tend to be present in several connective tissue planes, giving a distinctive staggered appearance (Plates 24, 25, 26, 27, 29). Horizontal sections demonstrate that the deeper and superficial vessels course parallel to the section with vertically oriented vessels (cut in cross section) present in all layers, but especially the deep and intermediate levels (Plates 26, 27, 29).

B. Laser Doppler Velocimetry

The prototype instrument of the TSI laser doppler was utilized to evaluate the capillary blood flow to the mucosal tissues along the buccal aspect of the posterior alveolus of the Rhesus monkey. For this experiment three time periods and three flap designs were utilized. Flap designs included: 1) tall narrow flaps (1:3) which were approximately 4mm wide and 12mm tall, 2) wide flaps (3:1) approximately 12mm wide and 4mm tall, and 3) envelope flaps approximately 20mm wide and 8mm tall. Doppler measurements were made pre-surgically, post-surgically following hemostasis, and post-suturing. Recordings were made at the margin, at the base of the flap and mid way between the two previous points at a tatoo location in the mesial-distal center of the flap (see Figure 2).

The two highest doppler readings obtained on successive recordings were annotated. Readings for each location were averaged and used in the statistical analysis of each site and time period. Data recorded with the laser doppler was annotated in real numbers calculated to correspond to ml flow/ (min x 100gm tissue). The baseline value for each site was the pre-surgical value. All other parameters (post-surgery, post-suture, and the percentage of baseline each represents) were compared against the

Plate 29

A. VASCULAR CORROSION CAST OF HORIZONTAL SECTION THROUGH THICK TISSUE (VIEWED FROM APICAL, SECTIONED IN KERATINIZED GINGIVA) (30.2x magnification). Capillary loops (c) are present along the epithelial surface. Arterioles are seen coursing primarily in a horizontal direction with some vertical branches (vf) along the entire width of the specimen.

B. H&E STAINED SPECIMEN OF THICK GINGIVAL TISSUE WITH SMALL HORIZONTAL AND VERTICAL VESSELS IN THE ENTIRE THICKNESS OF TISSUE (original magnification 10x). Larger vessels in this specimen are nearer the bone surface.

corresponding pre-surgical value. The mean value and standard deviation for each location (eg. margin of 1:3 flaps where n=6) were calculated at the three time periods and an analysis of variance was performed for statistical significance between baseline and postsurgical data. Postsurgical data was transformed into a percentage of the presurgical data for each site.

Results of doppler measurements are illustrated in Figures 8 through 12. Calculated percent of presurgical flow, means, and standard deviations for each flap design are included for each location (Appendix D).

As would be expected from descriptions of the vascular anatomy, following reflection and again following suturing, the margin of the narrow flap demonstrated a greater decrease in flow volume than either the wide or the envelope flaps. The margin of the narrow flap was the only one to demonstrate a significant change from presurgical values ($p = <.05$). Mean reductions (following reflection and suturing respectively) of 39.6 and 57.8 percent of the original flow were present in this region of the narrow flap. The wide flap maintained 55 and 81 percent of original flow volumes, and the envelope flap demonstrated 57 and 66 percent mean flow values when measured post flap reflection and post suturing.

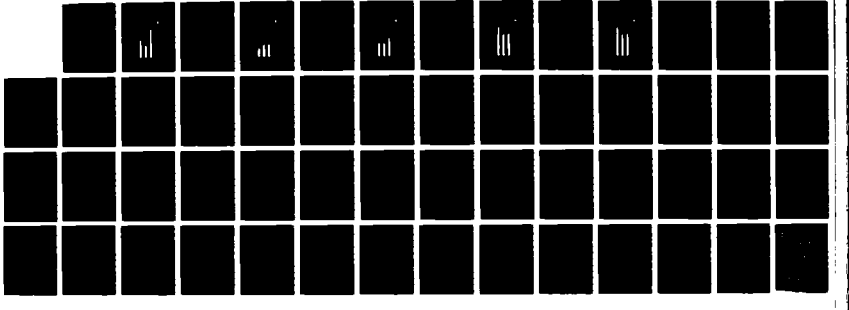
The middle portion of flaps demonstrated less impairment in vascular flow than the periphery. The base of the flaps demonstrated no flow reductions. Instead, the flap bases demonstrated an enhanced blood flow with mean values often exceeding baseline.

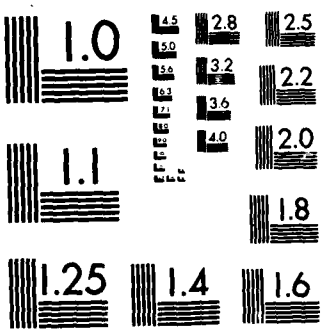
C. Fluorescein Angiography

Fluorescein angiography findings corroborated those reported by Mormann. Tall narrow flaps resulted in the most dramatic reduction in perfusion as demonstrated by this technique. The distal one half to two

Figure 8 MEAN BLOOD FLOW AT FLAP CORONAL MARGIN

AD-A196 199 VASCULAR PATTERNS AND PERFUSION OF MUCOGINGIVAL TISSUES 2/2
AND THEIR RELATIO..(U) AIR FORCE INST OF TECH
WRIGHT-PATTERSON AFB OH K A LINDELL MAY 87
UNCLASSIFIED AFIT/CI/NR-88-141 F/G 6/5 NL





MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

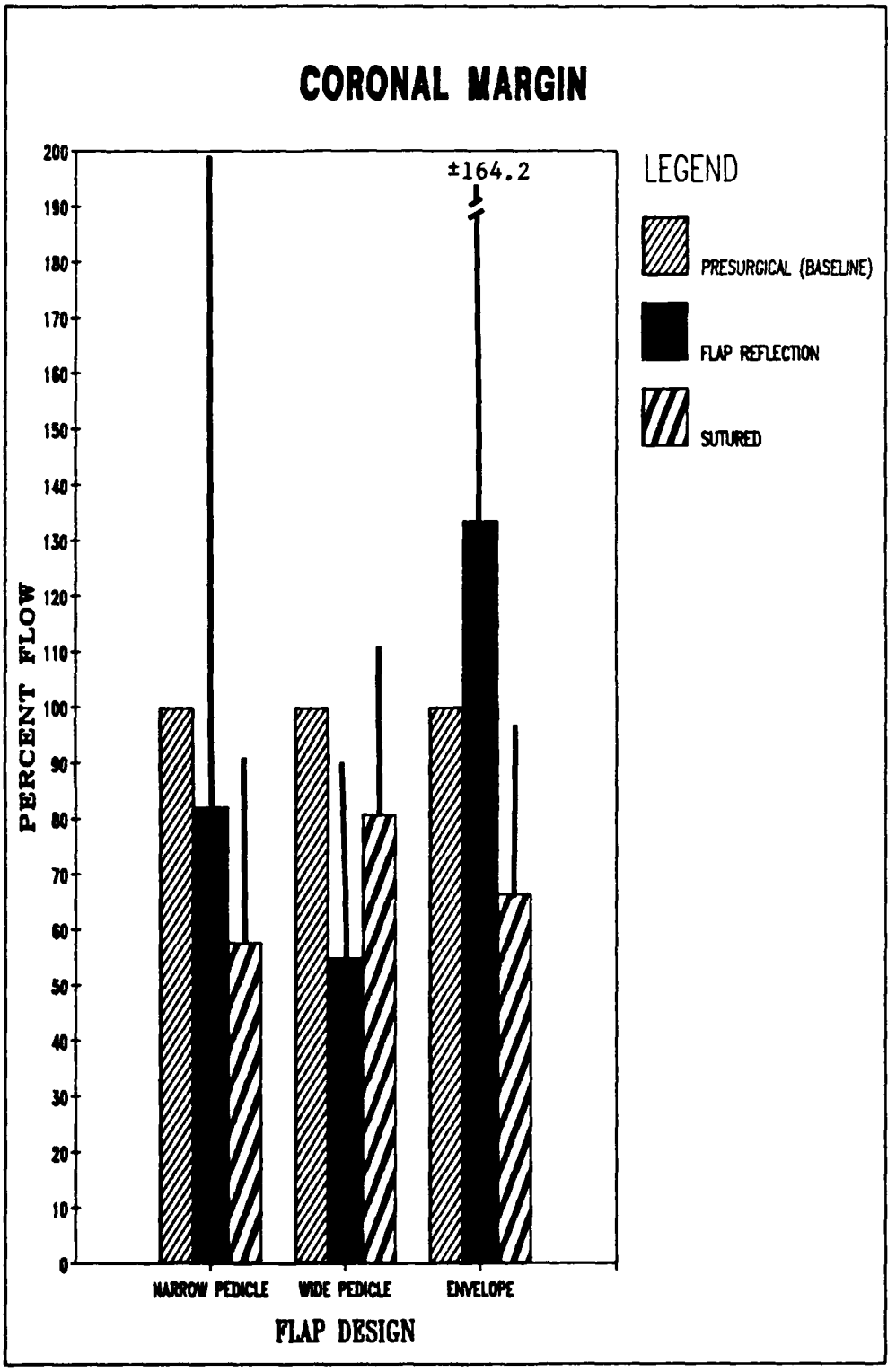

CORONAL MARGIN
±164.2
LEGEND
PRESURGICAL (BASELINE)
FLAP REFLECTION
SUTURED
PERCENT FLOW
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
NARROW PEDICLE
WIDE PEDICLE
ENVELOPE
FLAP DESIGN

Figure 9 ADJUSTED MEAN BLOOD FLOW AT FLAP CORONAL MARGIN

Mean percent blood flow to flap coronal margins following exclusion of two values approaching two standard deviations from the mean.

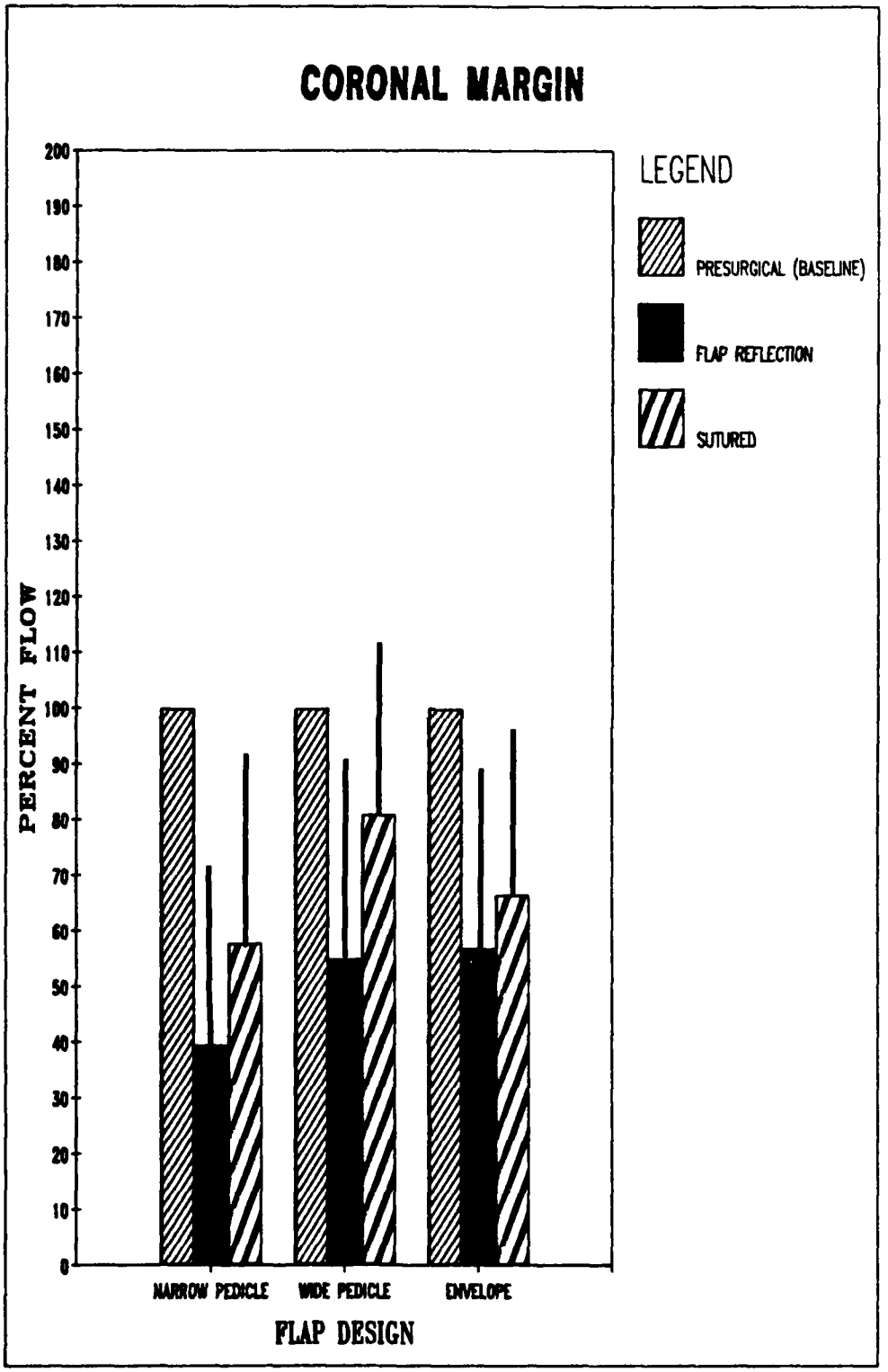

CORONAL MARGIN
LEGEND
PRESURGICAL (BASELINE)
FLAP REFLECTION
SUTURED
PERCENT FLOW
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
NARROW PEDICLE
WIDE PEDICLE
ENVELOPE
FLAP DESIGN

Figure 10 MEAN BLOOD FLOW AT FLAP MIDPOINT

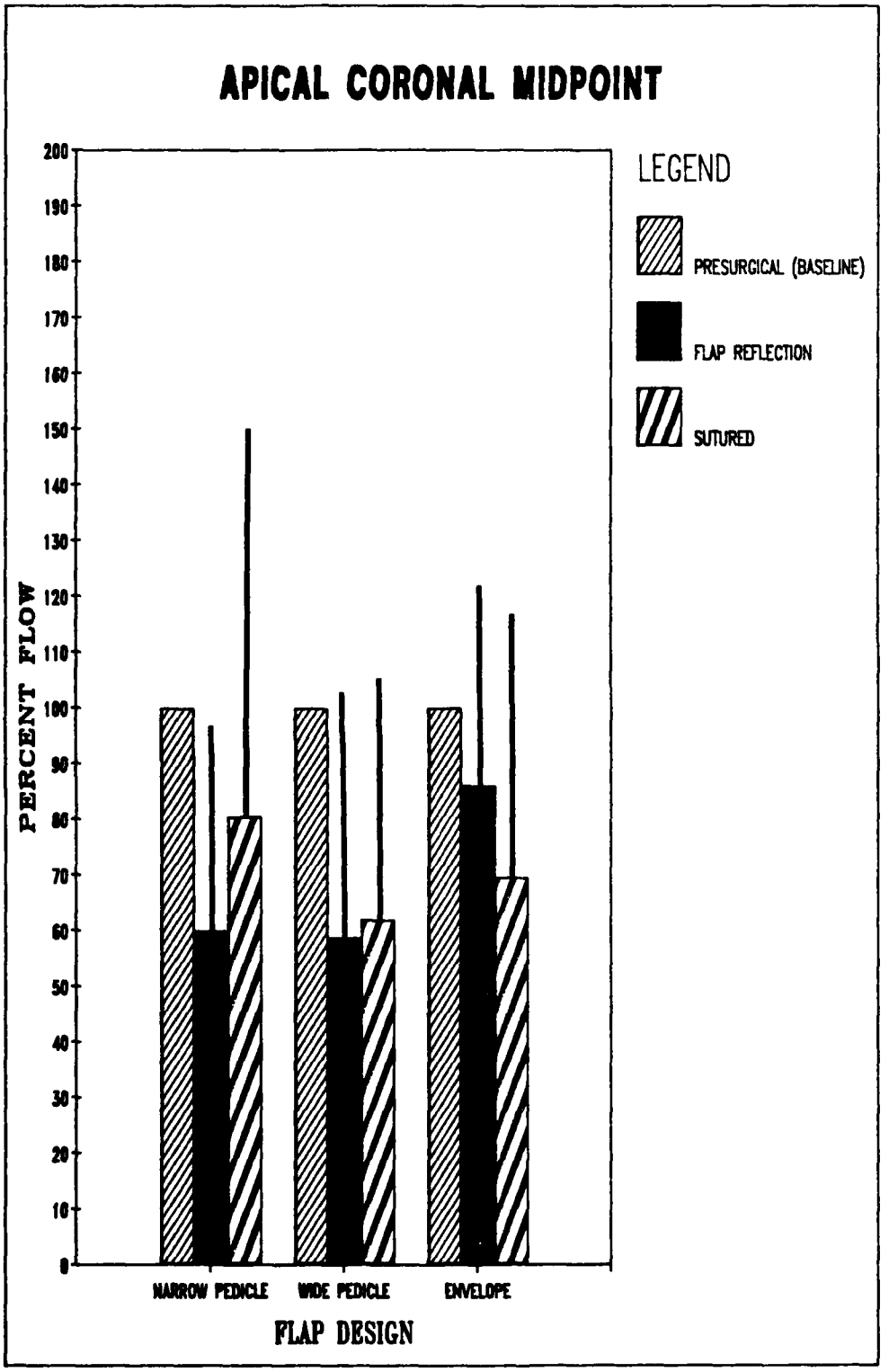

APICAL CORONAL MIDPOINT
LEGEND
PRESURGICAL (BASELINE)
FLAP REFLECTION
SUTURED
PERCENT FLOW
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
NARROW PEDICLE
WIDE PEDICLE
ENVELOPE
FLAP DESIGN

Figure 11 MEAN BLOOD FLOW AT FLAP BASE

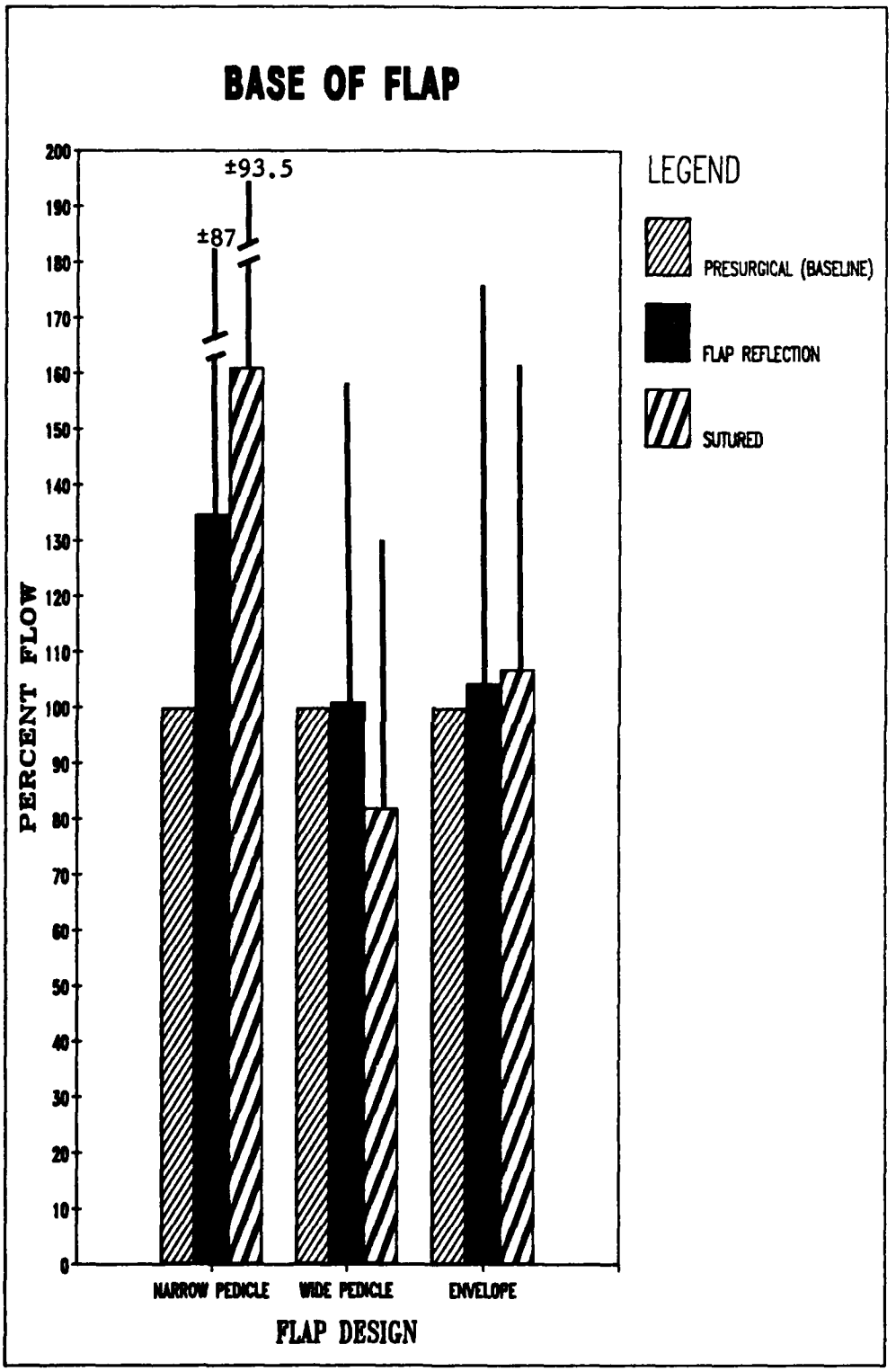
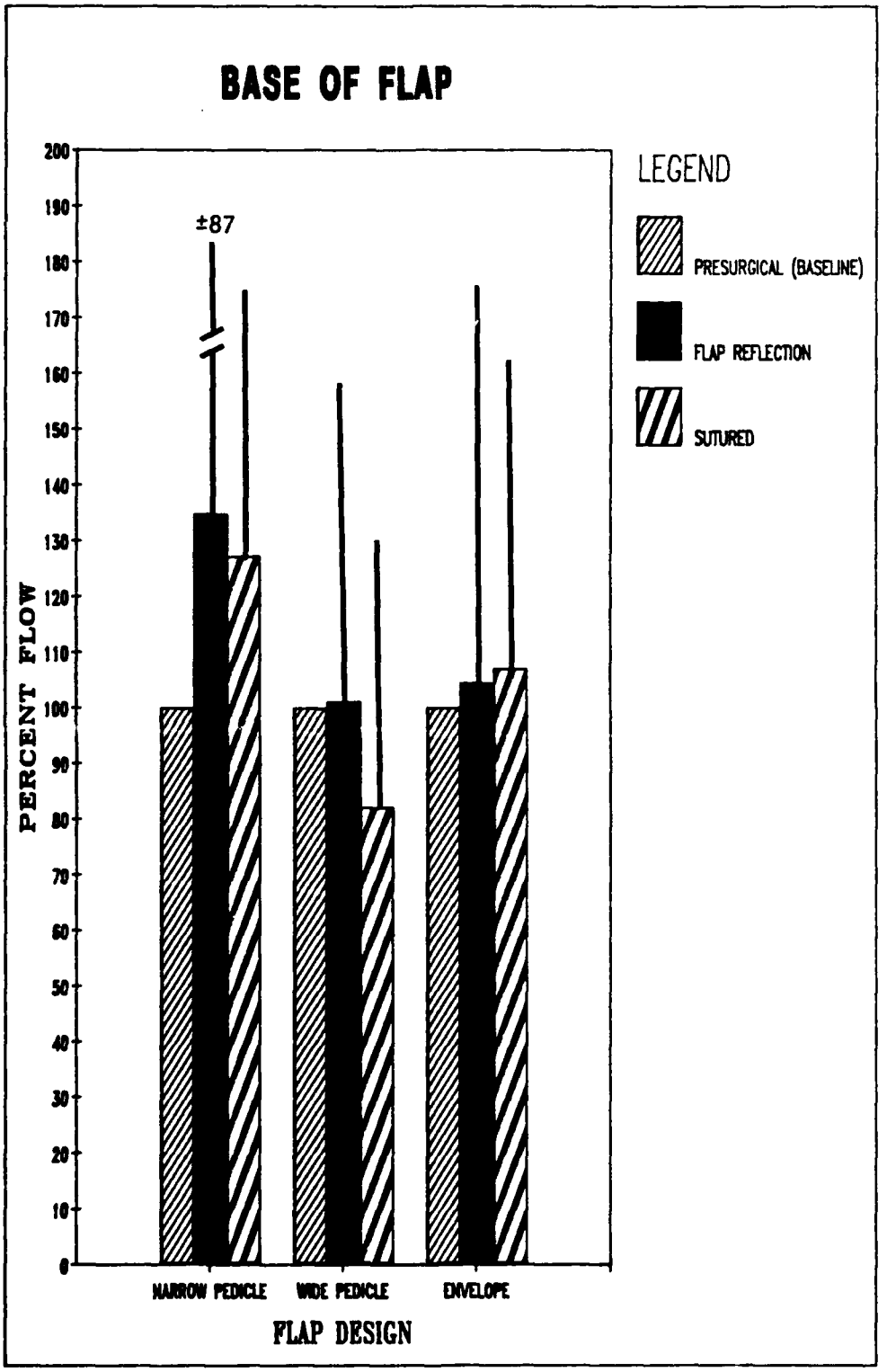

BASE OF FLAP
±93.5
±87
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
PERCENT FLOW
LEGEND
PRESURGICAL (BASELINE)
FLAP REFLECTION
SUTURED
NARROW PEDICLE
WIDE PEDICLE
ENVELOPE
FLAP DESIGN

Figure 12 ADJUSTED MEAN BLOOD FLOW AT FLAP BASE

Mean percent blood flow at the level of the flap base following exclusion of one value approaching two standard deviations from the mean.


BASE OF FLAP
LEGEND
PRESURGICAL (BASELINE)
FLAP REFLECTION
SUTURED
±87
PERCENT FLOW
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
NARROW PEDICLE
WIDE PEDICLE
ENVELOPE
FLAP DESIGN

thirds of the flap and the full extent of the vertical incisions demonstrated decreased fluorescence several minutes following intravenous injection of the dye. Wide pedicle flaps also demonstrated the decreased blood flow along the vertical incisions. Both wide pedicle and envelope flaps had non-perfused regions along the coronal margin, but to a lesser extent than that seen in the narrow flaps (which demonstrated fluorescence less than one half the vertical height).

Six days postsurgery two of six narrow flaps demonstrated necrosis (due to lack of perfusion), and both flaps were located near root prominences (Plate 30). Uneventful healing was noted in (1) those narrow flaps that included the entire papillary width, (2) in wide pedicle flaps and (3) in envelope flaps.

PLATE 30

A. FLUORESCEIN ANGIOGRAPHY OF ENVELOPE FLAP (e) IMMEDIATELY POST SURGERY AND HEALTHY SIX DAY NARROW PEDICLE (p).

B. FLUORESCEIN ANGIOGRAPHY OF ENVELOPE FLAP (e) IMMEDIATELY POST SURGERY AND NECROSIS OF MARGINAL THIRD OF SIX DAY NARROW FLAP (p).

VI. DISCUSSION

A. Techniques

The perfusion technique utilized in this study for Microfil and Batson's No 17 Corrosion Casting Compound was developed over several months through modifications of previously described techniques utilized in other organ systems. Perfusion of the oral soft tissues, especially with the very viscous corrosion casting material, proved to be unpredictable until the trendelenburg position was utilized. When the maxilla and mandible of the specimen were placed inferior to the cannulas in the common carotid arteries (by tilting the table to approximately 60 degrees), the casting material consistently filled the vessels of the head and face well. The use of warm saline rinses and a vasodilator prior to infusion also improved vascular fill.

Although the literature review for the Batson's No 17 Corrosion Casting Compound stated that the tissues would demonstrate better definition of individual cellular structures when the tissues were fixed prior to infusion of the material, prior fixation of the tissues proved to make the process of corrosion much more difficult and the bone surface tended to corrode at a rate equal to or greater than the fixed soft tissues, eliminating the support and references necessary for later processing. Since the need for support and references outweighed the need for definition of nuclear impressions and intercellular spaces, and since the study was more interested in the spatial relationships and orientation of the vessels themselves, prior fixation of the tissues was not utilized.

The use of corrosion casts allows a three dimensional study of the periodontal vasculature. This study is the first of its type to demonstrate the vasculature of the periodontal mucogingival tissues using vascular corrosion casts. The vasculature associated with areas of healthy tissues, with varying degrees of inflammation, as well as contrasting regions of thin and thick tissues were all evaluated. These phenomena are described in the posterior regions of non-surgerized, healthy Rhesus monkeys. Rhesus monkeys were selected for this study because: 1) the primate has been the model most frequently used in periodontal healing studies; 2) the anatomy of the monkey periodontium is very similar to that found in humans; 3) the size of the monkey periodontium almost approximates that of humans and permits an easier surgical and analytical manipulation of tissues, and 4) the Rhesus monkey has been shown to have the same major blood supply to the periodontal tissues (distal to the canine teeth) as that found in humans (Castelli, 1965). Since the posterior alveolar regions of the Rhesus monkey have been demonstrated previously to contain a vascular pattern similar to that found in humans, the information derived from this study should be applicable to the human subject. Other dissimilar animal models have been used in periodontal research. The rodent family has tooth and tissue relationships that are unlike the human and their diminutive size limits the operators ability to simulate human surgical procedures in healing studies. The dog model does not have the size problem, but differences in tooth and tissue morphology as well as an exceptional propensity for spontaneous healing present problems associated with interpolation of data. Also, the frequently encountered canine tissue pigmentation may interfere with healing assessment (personal communication with A. P. Shepherd).

The laser doppler has previously been utilized in human subjects to evaluate gingival tissues in healthy adults. The effects of tissue injury and healing have yet to be evaluated thoroughly with this instrument. This study is the first step in evaluating healing tissues through blood flow monitoring. The "time zero" blood flow information obtained following flap elevation and replacement and the effects of suturing were evaluated for trends and significance in three flap designs.

In another study a stent was used in an attempt to standardize the location of the doppler probe (Baab et al., 1986). The stent technique for probe placement was not utilized in this investigation because of post surgical positional differences in flap placement. Instead, a flap tatoo method was chosen so that the same flap location could be measured despite movement of flaps from their original position.

This study attempted to evaluate the highest consistent doppler readings in designated tissue regions, resulting in measurements of actual changes in flow following tissue manipulation. The "best case" scenario has already been evaluated by utilizing pre-surgical recordings of the lowest repeatable values followed by post surgical recordings of the highest repeatable values (Moskowicz, personal communication, unpublished data). A "worst case" scenario utilizing the doppler should provide better prognostic information regarding flap survival.

The use of the probe tip available with the prototype TSI laser doppler instrument made exact repositioning (at different times) difficult because of its large size (3 mm diameter). The probe was positioned in this study with the fiberoptic bundles oriented along the apical-coronal line of the flaps to aid in decreasing measurement error. A smaller probe tip is now

available (0.8 mm diameter) from TSI Inc. and its use (in areas such as the oral soft tissues) is recommended for more accurate doppler placement.

Extremely high doppler readings (well beyond the average range) may be a result of placement of the fiberoptic probe tip over a major blood vessel (such as an apical-coronal feeder vessel). When the probe tip was moved slightly to a new location, the reading often-times dropped dramatically to parallel the average value of other measured readings.

Three of the tatoo recordings resulted in either post surgical or post suture percentages that exceeded two standard deviations above the mean presurgical value. These exceedingly high values could possibly be due to 1) placement of the probe tip over a major vessel not previously recorded or 2) movement of the probe or 3) movement of the animal during the recording session. By eliminating these exceptionally inflated values (ie. those > 2 standard deviations from the mean) and by recalculating data and mean values (for flap manipulation and tatoo location), definite trends can be seen.

B. Anatomic Findings

1. Periodontal Ligament

The apical-coronal prominence of the periodontal ligament vasculature (as demonstrated in H&E and cleared sections) confirmed the findings of Carranza et al. (1966), but they were unable to consistently verify that the position of the vascular plexus was nearer to the osseous surface than to the tooth. Instead, the bulk of vascular elements were seen around the midpoint of the periodontal ligament (Plates 3,4). The surprising prominence of this plexus could be due to the use of a vasodilator (prior to fixation of the tissues) to enhance Microfil perfusion. Interestingly, vascular casts of gingiva, where

the same vasodilator was used prior to perfusion, resulted in casts with capillary loops ranging from 6-15um in lumen diameter (in health); in regions of inflammation, intermediate size vessels and dilated capillaries ranged from 20-70um in diameter and larger feeder vessels to the gingival margin were 90-140um in diameter. These ranges are within values previously reported (Hansson et al, 1968, Waerhaug, 1952, Nuki and Hock, 1975).

The flat plexus of vessels within the periodontal ligament appear to course the entire length of the ligament space and extend into the sulcular area where they closely parallel healthy sulcular epithelium. These vessels can be demonstrated not only in the H&E sections but also in some of the corrosion casts where they appear as a continuous flat network of similar sized (6 - 10 um diameter) vessels with many anastomoses. (Plates 2, 9, 10)

2. Periosteum

Evidence of a very dense layer of periosteal capillaries adjacent to bone was found in both the corrosion casts and histologic specimens. The appearance of this dense layer of vessels beneath a comparatively avascular reticular fiber layer corresponds to the layers in the periosteum demonstrated and described by Cutright (1980) in latex perfused specimens.

3. Free Gingival Margin

As the healthy gingival margin is approached, loop formations are consistently seen in the sulcular aspect of the free gingival margin. Vascular casts graphically demonstrate that the vascular loops in this

region are slightly larger in diameter and more twisted than those seen on the oral surface (Plates 10, 11). These vascular loop formations and the flat sulcular plexus were previously described from serial sections of ink perfused canine gingiva (Egelberg 1966) and with vital microscopy (Hansson et al., 1968).

As inflammation increased, H&E sections demonstrated increased proliferation of the epithelial lining of the sulcus with the formation of irregular ridges with interposed connective tissue extensions containing engorged and dilated capillaries (Plates 8, 11, 12). Increasing inflammation was associated with increasing proliferation of epithelium, more tortuous capillary loops, and larger numbers of inflammatory cells, primarily plasma cells. The inflammatory vascular proliferation in this region was previously described by Hansson et al (1968), utilizing vital microscopy, and Kindlova (1965) who described "glomerular-like" capillary loops subsequent to latex perfusion of the vasculature. The remarkable changes in length and diameter of capillaries with increasing inflammation, as described by Nuki and Hock (1975), as well as capillary dilations and varicosities, were unequivocally demonstrated in vascular casts in this study (Plates 9, 10, 11, 13). In addition, as the degree of inflammation increased, these vessels extended further into the sulcular area (Plates 8, 9, 10, 11, 13, 17).

In agreement with Nuki and Hock (1975) and Egelberg (1966), the sulcular aspect of the marginal gingiva was the first to demonstrate vascular changes due to inflammation (Plates 8, 9, 10, 11), followed soon after by deeper vascular extension into the sulcus (Plates 12, 13,

17), and only much later could changes be seen on the oral aspect of the tissues (Plate 12).

4. Surface Capillary Configuration

The superficial blood vessels associated with the oral surface of the epithelium are also of capillary size (6 - 15 um diameter). The orientation of these vessels differs dramatically in the regions of the attached and free gingiva as compared to the alveolar mucosa region apical to the mucogingival junction (Plate 14). Cleared sections demonstrate the dense nature of the capillaries of the lamina propria when the tissue is sectioned either vertically or horizontally (Plate 15, 26). Vascular corrosion casts demonstrate these vessels even more dramatically; individual vascular loops are easily identified along the superficial aspect of the specimens with a straight, narrow, hairpin appearance (Plates 9, 10, 14, 15). Confirming additional findings of Nuki and Hock (1975), this study also demonstrated that as the free gingival margin is approached, the capillary loops are longer and more numerous than in the more apical regions of the keratinized tissues (Plates 9, 10, 14, 15). Egelberg (1966) and others have suggested that it is probably the proximity to plaque and the subsequent development of a mild chronic inflammation that results in the increased number and length of capillary loops in this region, as well as the depth of epithelial proliferation.

An abrupt change in superficial capillary configuration is present at the mucogingival junction (Plates 14, 15). The hairpin loops disappear and are replaced by a flattened network of short loops with a slightly twisted appearance. The diameter of these vessels is

comparable to the capillary loops in the keratinized tissues but their length is greatly decreased.

5. Major Vessel Orientation in Keratinized Gingiva

Subjacent to the capillary loops a flat, thin layer of small capillaries, venules, and arterioles is present in both the mucosal tissues and the keratinized tissues of the attached and free gingiva. The deeper layers of the connective tissues contain the major blood vessels and demonstrate definite patterns of orientation depending on location and tissue thickness. Keratinized tissues appear to have a predominance of medium sized horizontal vessels, which course parallel to the mucogingival junction. Branches from these vessels form the more superficial layers and capillary loops. This vessel orientation is seen on vascular casts from both the oral and periosteal surfaces (Plates 9, 15, 21). Vertical sections also demonstrate the cross sections of cut horizontal blood vessels (Plates 8, 11, 12, 13, 21, 24). This finding confirmed that of Nuki and Hock (1975) in that they describe the arterioles as coursing parallel to the gingival margin while giving rise to branches that eventually form capillary loops along the epithelium.

As the gingival margin is approached, vertically oriented vessels can be seen especially in thicker tissue regions (ie. papillae and furcations) (Plates 9, 11, 18, 19, 20, 21). The vertically coursing vessels in these regions branch and "fan" to supply adjacent areas and the gingival margin. Areas of thinner tissues, located over root prominences, seldom demonstrated the vertically oriented vessels; instead, horizontal vessels supply this region and branch to form the

capillary loops of the free gingival margin and overlying keratinized epithelium. Kindlova (1965) described the vertically oriented vessels supplying the gingiva as coursing apical-coronally along the bone and giving off superficial branches to the epithelium. His diagrams depict this finding. This study demonstrated a predominance of horizontally and obliquely coursing vessels with some branches oriented vertically, (especially prominent in the papillary regions of the gingiva and the thicker regions of the mucosa (Plates 21, 23, 26)). Orientation of major vessels in the gingival tissues appears to be correlated with the thickness of the connective tissues.

6. Tissue Thickness and Vessel Orientation

Gingival tissue thickness in humans was described by Goaslind et al. (1977). They reported that: 1) the average gingival thickness at the base of the sulcus was 1.56 $\pm$ 0.39 mm; 2) the average thickness of the attached tissue was 1.25 $\pm$ 0.42 mm; 3) the thickness of free and attached tissues increased from anterior to posterior regions in the mandible and remained nearly constant in the maxilla. Thickness of the mucosal tissues was not evaluated. Epithelial thickness has been described in human palatal tissues to be .11 - .62 mm in thickness with an average of .36 mm (Soehren et al, 1973). If the same mean thickness of epithelium can be extrapolated to the facial and buccal tissues of the periodontium, a periodontium may contain as little as 0.47 mm or as great as 1.31 mm of connective tissue thickness in attached gingiva. A similar variation in thickness can also be found in the mucosal tissues apical to the mucogingival junction.

In this study, thickness of tissue could not be correlated well with specific areas of the periodontium since both thin and thick tissues were found in both molar and premolar regions of the four animals. In general, thicker regions demonstrated an increase in the total number of blood vessels, a large proportion of these vessels coursed in a horizontal or oblique direction, and vessels appeared to be located in tissue planes that were easily separated. Similar to Goaslind's findings, the base of the sulcus was associated with thicker tissue than apical areas in attached gingiva. The thinnest region of connective tissue was consistently found to be at the level of the mucogingival junction. (Plates 21, 22, 24, 28)

A vascular "palisading effect" was previously described (Kindlova, 1965) in the marginal tissues where groups of major vessels branched to form smaller arterioles that subsequently branched into more superficial layers to form capillary loops (Plates 8, 9, 11, 29). The present finding of a layering of vessels in more apical thicker tissues (Plates 22, 23, 25, 26, 27) has not been previously described. The vascular layering found in thick tissues and not seen in thin tissues, probably accounts for more predictable flap survival of partial thickness flaps since the reflected flap and the residual stationary tissues both contain an intact major blood supply.

Previous studies have recommended no greater than a 1:2 (width to height) flap ratio to ensure an adequate apical blood supply to the pedicle (Ohmori and Kurata, 1960). Patterson and Chir (1968) also recommended a similar ratio with skin flaps, but found that the inclusion of a major vessel to the flap allowed for the design of a greater flap length. This information can be extrapolated to the

mucogingival tissues and demonstrated clinically. An area containing a major feeder vessel coursing the length of the pedicle will help maintain the blood supply even to coronal margins of flaps longer than the 1:2 ratio. The tall narrow pedicle flaps utilized in double papilla flap procedures can approach the 1:4 width to height ratio. The selection of relatively thick adjacent interproximal tissues that contain larger vertically oriented feeder vessels can ensure survival of tall pedicle flaps (even when eventually positioned over avascular root surfaces).

7. Fluorescein Angiography Studies and Their Relation to Vascular Orientation

The reported apical to coronal blood supply found in the mucogingival tissues by Mormann and Ciancio (1977) can be accounted for even in the presence of a large predominance of horizontally oriented vessels. Mormann and Ciancio studied reductions of vascular perfusion in gingival tissues coronal to a horizontal incision. They found normal perfusion, as demonstrated by fluorescein dye, in locations primarily at or near the interproximal regions, and reduced perfusion near line angles and over root prominences. This data would correspond well with findings in this study in that following regional interruption of flow to an area, larger vertically oriented vessels should reperfuse more quickly than similar regions with smaller horizontally oriented arterioles and capillaries. Recall that the major blood supply to the marginal area came from large vertical vessels in the interproximal region.

When pedicle flaps were evaluated by Mormann and Ciancio (1977), vertical incisions demonstrated a disturbance in circulation along the incision line, even 24 hours following injury. This can be explained by recognizing that horizontal and/or oblique vessels were interrupted in the region; the severed vessels retracted, a clot was formed but minimal endothelial proliferation occurred at 24 hours. The initial stage of clotting is termed the vascular phase because the injured vessels contract and retract from the injury site, and thus leave the immediate incision area ischemic.

Following flap elevation, Mormann described a 50% reduction in perfusion, as estimated by fluorescein angiography. Not only were the vertical incision lines poorly perfused, but in addition, a large reduction in fluorescein perfusion was noted in the center of the flap. The blood flow into the few vessels coursing coronally did not have sufficient hydrostatic pressure to perfuse beyond the central portion of the flap, leaving the coronal and lateral aspects of the flap poorly perfused. The vascular supply to the center of the elevated flap was likewise decreased (to a lesser extent) because of the overall reduction in hydrostatic pressure resulting from the loss of communicating arterioles severed from underlying bone.

The long narrow flap in the Mormann study resulted in necrosis along the vertical incisions, possibly due to their location in thin tissues over root prominences where the vessels are smaller, and rely on collateral blood supply from the larger vessels located in the interproximal regions. Radicular areas have thinner bone and smaller perfusing arterioles that arise from the periodontal ligament. Similar findings were noted in this study (Plate 30).

This investigation also evaluated the length to width ratio of pedicle flaps and found that wider flaps had better perfusion and healing than long narrow flaps. The coronal and lateral margins of both flap designs were compromised, but to a much greater extent when a long flap design was utilized. Since the blood supply to the flap is from the apical aspect, the width of the flap base limits the number of apical to coronal vessels and the potential for optimal perfusion. The coronal portion of the flap as well as the lateral periphery of the flap adjacent to vertical incisions were poorly perfused as was demonstrated with fluorescein angiography. This finding correlates well with the porcine skin flap study by Patterson and Chir (1968) where the most distal portion of flaps often required more than 30 minutes to perfuse and when this occurred, the poorly perfused regions eventually necrosed. This same phenomenon can occur intraorally when a flap lacks good perfusion to the most coronal and lateral aspects (as was seen in some of the narrow pedicle flaps (Plate 30)). In 2 of 6 specimens, the coronal one fourth of the flap failed to survive and the necrotic area was replaced by granulation tissue 6 days post surgery.

8. Doppler Results and Relation to Vasculature

The doppler readings of the narrow flap immediately after reflection also demonstrated a significant decrease in perfusion of the most coronal aspect (39 % of original flow) with a subsequent increase (to 57%) following suturing. The immediate and initial low perfusion followed by slow increase over time was the same phenomenon described by Patterson. This phenomenon of increased flow over time is related to an increased peripheral resistance once clotting begins along the

incised flap margins, which leads to improved hydrostatic pressure within the vessels and a resultant increased blood flow to more coronal aspects of the pedicle. Wide pedicle and envelope flaps demonstrated the same phenomenon, but to a lesser extent. Flaps designed with a wider base contain more vertically oriented vessels and allow more blood to rapidly reach the most coronal regions, therefore recordings of wide flaps immediately post surgery did not drop as dramatically from baseline values. The decrease in perfusion that occurs following flap incisions is probably due to a combination of factors including: 1) bleed-out phenomenon along the cut margins due to decreased peripheral resistance, 2) vascular retraction and clot formation leading to area ischemia, and 3) severing of underlying communicating vascular channels from periosteum and bone. The enhanced blood flow noted at the flap base may be due to an increase in the bypass flow rate of blood due to decreased peripheral resistance resulting from severed vessels.

Although this study evaluated post-surgical perfusion to the flaps, it did not determine the delayed reperfusion of tissue and its relation to flap survival. The predictive capability of flap survival (as measured with laser doppler velocimetry) increases up to 24 hours post surgery (Larrabee et al., 1983, Marks et al., 1984). Short term projections of flap survival suffer from error measurements caused by the oscillating motion of the red blood cells or the bypass flow through anastomosing vessels which do not actually represent nutritive flow to the tissues (Liu et al., 1986).

C. Clinical Implications

Findings of this study indicate that either wide based pedicle or envelope flaps have a predictable healing result when utilized as full thickness flaps. Other flap designs vary in predictability depending on tissue thickness and major blood vessel orientation.

Because of the occurrance of major vertically oriented vessels in interradicular regions, vertical incisions for relaxation of a mucogingival flap should be made to include the entire papilla (to the nearest line angle of the adjacent tooth not included in the flap). This design also gives the added benefit of greater bulk for suturing and a decreased likelihood of impairing vascular perfusion in radicular regions of the flap. A similar rationale for placement of vertical relaxing incisions can be applied to the palate when removing a connective tissue graft or any other procedure requiring a vertical incision in the region. If the vertical incision is made only at the most mesial aspect of the flap (for access to the the underlying tissues), the posterior to anterior major vasculature to the remainder of the flap will not be compromised. Whereas a vertical incision along both mesial and distal margins can result in necrosis of the entire flap and resultant healing by secondary intention.

The degree of tissue thickness must be taken into consideration in dealing with soft tissue procedures and should help dictate flap design. Tissue thickness will determine the number of perfusing vessels and thus can alter the maximum width to height ratio for flap survival. Thick tissues can also be utilized for split thickness flaps, whereas thin tissue cannot.

Suture placement and tension must also be considered. A suture placed near an incision line may additionally compromise an already reduced perfusion by adding tension and increasing resistance to flow. It was

demonstrated by Mormann and Ciancio that increased tension on the flap will impair vascular perfusion, and research presently being performed (Moskowicz, unpublished) demonstrates a 5-15% reduction in flap perfusion following suture placement. Thus, sutures should be placed away from poorly perfused areas, at least 1 1/2 mm from margins (possibly 2 mm with beveled margins), and suture tension should be minimized.

VII. SUMMARY

This study was undertaken 1) to demonstrate the normal vascular anatomy of the buccal periodontal tissues of the Rhesus monkey and the spatial relationship of the vessels according to location and tissue thickness, and 2) to evaluate tissue blood flow following three flap designs as recorded with non-invasive laser doppler velocimetry and fluorescein angiography. Four animals were sacrificed following perfusion of head and neck vasculature with silicone Microfil or Batson's #17 corrosion casting medium. Microfil perfused sections of posterior alveolar regions were prepared for H&E staining or cleared sections, and Batson's perfused specimens were prepared for scanning electron microscopy.

Vascular corrosion casts corroborated the findings of previously utilized techniques. Changes in sulcular capillaries consisting of increases in length and diameter associated with inflammation, as well as changes in capillary configuration from attached gingiva to alveolar mucosa were recorded. These findings have been previously described by several authors (Nuki and Hock (1975), Hansson et al. (1968), Kindlova (1965)). A new finding reported in this study was the observation that vascular relationships change as tissue thickness changes. As tissue thickness increased, the vascular supply appeared to organize in distinct tiers. A predominance of large vessels (coursing in a horizontal or oblique direction) was noted in alveolar mucosa with fewer branches coursing vertically to supply more coronal tissues. The more apical tissues near the fornix of the vestibule had a less well defined vascular orientation. In attached gingiva, a bed of communicating horizontal vessels seems to predominate and appears to be interrupted by vertically oriented feeder vessels in thicker tissues associated with interradicular areas (to include

furcal regions). Branches of the vertically oriented vessels in the attached gingival tissues help form the horizontal mat of vessels previously described.

The laser doppler evaluation of tissue blood flow associated with surgical flap design has not previously been utilized to study periodontal tissues. Changes in tissue perfusion could be seen with surgical manipulation of flaps. This study demonstrated a significant decrease in tissue perfusion in the coronal aspect of tall narrow flaps (1:3 ratio) with resultant marginal flap necrosis in 2 of 6 flaps. Less reduction in perfusion was seen in wide (3:1 ratio) and envelope flaps. All designs demonstrated an enhanced blood flow at the base of flaps. The impairment in vascular flow varied according to tissue thickness, location and number of apical-coronal feeder vessels, suture tension, time of doppler recording following flap manipulation (ie. flow improved following clot formation), and distance from flap base to coronal and lateral margins.

VIII. CONCLUSIONS

1. Major blood vessel orientation of the mucogingival tissues in the regions distal to the canine teeth in the Rhesus monkey is dependent upon 1) specific location and 2) tissue thickness.

2. A larger proportion of horizontally and obliquely oriented blood vessels are present in gingiva and mucosa than previously reported.

3. Vertically oriented vessels in the gingival tissues are almost exclusively located in interradicular regions where tissues tend to be thicker. Other areas of gingiva contain smaller and more horizontally oriented vessels.

4. Thick mucosal tissues contain a tiered arrangement of blood vessels not seen in thin tissues. The tiers contain both horizontal and vertical vessels, with the largest vessels localized in the deepest layers (near bone).

5. Laser doppler velocimetry demonstrated a postsurgical decrease in vascular flow at the margin and midpoint of the flap. However, the reduction in perfusion was statistically significant only for the marginal area of narrow flaps with a width to height ratio of 1:3.

6. Increase in area perfusion (to the marginal areas of the flap) was noted over time, a phenomenon also seen previously in skin flaps and is probably related to clot formation and the reestablishment of hydrostatic vascular pressures.

7. Doppler recordings at the base of flaps demonstrated an enhanced blood flow post surgically.

IX. RECOMMENDATIONS FOR FURTHER STUDIES

Laser doppler technology opens up a new field of non-invasive study related to tissue healing. This instrument should continue to be used for evaluating tissue perfusion during various phases of surgery and healing with an ultimate goal of obtaining more reliable prognostic information regarding flap designs and survival. Other possible applications of this instrument could include the evaluation of blood flow changes secondary to medicinal usage, systemic disease, and post radiation changes.

Appendix A

H&E Prepared Specimens

H&E SPECIMENS

Animal number	Histo label	Location of Origin	Section orientation
640 C	85-087 A	Facial Maxilla, 2 premolar - 2 molar	horizontal
640 C	85-087 B	Facial Maxilla 1 premolar	frontal
640 C	85-087 C	Facial Maxilla 2 & 3 molars	frontal
640 C	85-087 D	Palate 2 premolar - 2 molar	horizontal
640 C	85-087 E	Palate 1 premolar	frontal
640 C	85-087 F	Palate 2 & 3 molars	frontal
640 C	85-087 G	Mandibular 1 premolar - 1/2 of 2 premolar	horizontal
640 C	85-087 H	Mandibular 2 premolar- 1 & 2 molar	frontal
640 C	85-087 I	Mandibular 2 & 3 molars	horizontal
127 D	85-088 A	Facial Maxilla 2 premolar - 2 molar	horizontal
127 D	85-088 B	Facial Maxilla 1 premolar - 2 premolar	frontal
127 D	85-088 C	Facial Maxilla 2 & 3 molars	frontal
127 D	85-088 D	Palate 2 premolar - 2 molar	horizontal
127 D	85-088 E	Palate 1 & 2 premolar	frontal
127 D	85-088 F	Palate 2 & 3 molars	frontal
127 D	85-088 G	Mandibular 1 premolar	frontal
127 D	85-088 H	Mandibular 2 premolar - 1 molar	horizontal
127 D	85-088 I	Mandibular 2 & 3 molars	frontal

Appendix B

Cleared Sections

CLEARED SECTIONS

animal	block	location			direction of sections
640 C	A	Facial	Max	2 premolar-2 molar	horizontal
640 C	B	Facial	Max	1 premolar	vertical
640 C	C	Facial	Max	2 molar-3 molar	vertical
640 C	D	Palatal	Max	2 premolar-2 molr	horizontal
640 C	E	Palatal	Max	1 premolar	vertical
640 C	F	Palatal	Max	2 molar-3 molar	vertical
640 C	G		Man	1 premolar-1/2 2 premolar	horizontal
640 C	H		Man	2 premolar-2 molar	vertical
640 C	I		Man	2 molar-3 molar	horizontal
127 D	A	Facial	Max	2 premolar-2 molar	horizontal
127 D	B	Facial	Max	1 premolar-2 premolar	vertical
127 D	C	Facial	Max	2 molar-3 molar	verticaL
127 D	D	Palatal	Max	2 premolar-2 molar	horizontal
127 D	E	Palatal	Max	1 premolar- 2 premolar	vertical
127 D	F	Palatal	Max	2 molar-3 molar	vertical
127 D	G		Man	1 premolar	vertical
127 D	H		Man	2 premolar-1 molar	horizontal
127 D	I		Man	2 molar-3 molar	vertical

Appendix C

Vascular Corrosion Cast Specimens

CORROSION CAST SPECIMENS

Animal	Sample number	Location of Section
886 C	1	mandibular left facial 3rd molar
886 C	2	mandibular left interprox 2-3 molars
886 C	3	mandibular left facial 2 molar
886 C	4	mandibular left facial 1 molar
886 C	5	mandibular left interproximal of premolars
886 C	6	mandibular right facial 1 premolar
886 C	7	mandibular right premolar interproximal and 2 premolar
886 C	8	mandibular right molar- premolar interproximal, 1 molar
886 C	9	mandibular right interprox 1-2 molar, 2 molar facial
886 C	10	mandibular right facial 2 molar, proximal 2-3 molars
886 C	11	maxillary right 2-3 molar interproximal
886 C	12	maxillary right facial 2 molar, proximal 1-2 molars
886 C	13	maxillary right 1 molar facial (dehiscence)
886 C	14	maxillary right mid 2 pre - mid 1 premolar
886 C	15	maxillary right 1 premolar - edentulous
886 C	16	maxillary right proximal lateral-central
886 C	17	maxillary left 2-3 molar interproximal
886 C	18	maxillary left 2 molar facial
886 C	19	maxillary 1-2 molar interproximal
886 C	20	maxillary left 1 molar facial (dehiscence)
886 C	21	maxillary left facial 2 premolar, proximal molar-premolar
886 C	22	maxillary left premolar proximal, 1 premolar (dehiscence distal)
178 D	31	maxillary right 2-3 molar
178 D	32	maxillary right 1-2 molar interproximal + vestibule
178 D	33	maxillary right 1 molar, molar-premolar proximal
178 D	34	maxillary right 1-2 premolar proximal (v. thin)
178 D	35	maxillary right 1 pre - edentulous
178 D	36	maxillary left 2-3 molar interproximal
178 D	37	maxillary 1-2 molar proximal
187 D	38	maxillary left 2 premolar
187 D	39	maxillary left 1 premolar
178 D	40	mandibular right 2 molar
178 D	41	mandubular right 1-2 molar interproximal
178 D	42	mandibular right 1 molar furca + mesial proximal
178 D	43	mandibular right 1-2 premolar proximal, 2 premolar
178 D	44	mandibular right 1 premolar
178 D	45	mandibular left 1-2 premolar interproximal
178 D	46	mandibular laft 1 premolar
178 D	47	mandibular left 2 premolar - 1 molar furca
178 D	48	mandibular left 3 molar

Appendix D

Doppler Data

MARGIN NARROW FLAP

animal	location	pre	post-surgery	%	post-suture	%
572	max	.04	.13	325	.05	125
572	man	.11	.05	45	.03	27
992	max	.13	.08	61	.10	76
992	man	.25	.10	40	.13	52
110	max	.18	.04	22	.04	22
110	man	.20	.06	30	.09	45
mean		.15167	.07667	82.2	.07333	57.8
SD		.07414	.03386	117.3	.03933	33.8
Tail probability		.0281				
Greenhouse-Geisser		.0637				
Huyhn-Felt		.0583				
adjusted mean				39.6		57.8
SD				33.1		33.8

MIDDLE NARROW FLAP

animal	location	pre	post-surgery	%	post-suture	%
572	max	.68	.12	17	.11	16
572	man	.31	.08	25	.35	112
992	max	.24	.14	58	.08	33
992	man	.36	.43	119	.73	202
110	max	.20	.13	65	.16	80
110	man	.94	.72	76	.38	40
mean		.45500	.27000	60	.30167	80.5
SD		.29228	.25424	36.9	.24408	69
Tail probability		.2679				
Greenhouse-Geisser		.2744				
Huyhn-Felt		.2721				
adjusted mean				60		80.5
SD				36.9		69

BASE NARROW FLAP

animal	location	pre	post-surgery	%	post-suture	%
572	max	.21	.54	257	.36	171
572	man	.28	.19	67	.19	67
992	max	.35	.47	134	.62	177
992	man	.36	.43	119	.46	127
110	max	.35	.85	242	1.16	331
110	man	.87	.44	50	.82	94
mean		.40333	.48667	134.8	.60167	161.1
SD		.23577	.21379	87	.34862	93.5
Tail probability		.2929				
Greenhouse-Geisser		.2946				
Huyhn-Felt		.2929				
adjusted mean				134.8		127.2
SD				87		47.8

MARGIN WIDE FLAP

animal	location	pre	post-surgery	%	post-suture	%
572	max	.15	.16	106	.08	53
572	man	.14	.12	85	.15	107
992	max	.13	.04	30	.15	115
992	man	.12	.10	83	.11	91
110	max	.08	.09	112	.07	87
110	man	.18	.05	27	.07	38
mean		.13333	.09333	55.1	.10500	81
SD		.03327	.04457	36.9	.03782	31.1
Tail probability		.2569				
Grenhouse-Geisser		.2585				
Huyhn-Felt		.2569				
adjusted mean				55.1		81
SD				36.9		31.1

MIDDLE WIDE FLAP

animal	location	pre	post-surgery	%	post-suture	%
572	max	.29	.10	34	.13	44
572	man	.13	.10	76	.17	130
992	max	.48	.14	29	.44	91
992	man	.40	.57	142	.50	125
110	max	.67	.15	22	.13	19
110	man	.16	.08	50	.15	93
mean		.35500	.19000	58.8	.38667	62
SD		.20482	.18804	45	.31992	44.2
Tail probability		.3781				
Greenhouse-Geisser		.3659				
Huyhn-Felt		.3766				
adjusted mean				58.8		62
SD				45		44.2

BASE WIDE FLAP

animal	location	pre	post-surgery	%	post-suture	%
572	max	.50	.59	118	.33	66
572	man	.32	.57	178	.43	134
992	max	1.31	.47	35	.54	41
992	man	.90	1.38	153	1.33	147
110	max	.58	.45	77	.40	68
110	man	.69	.32	46	.26	37
mean		.71667	.63000	101.1	.54833	82.1
SD		.34909	.37995	58.1	.39443	47.1
Tail probability		.5551				
Greenhouse-Geisser		.4734				
Huyhn-Felt		.4795				
adjusted mean				101.1		82.1
SD				58.1		47.1

MARGIN ENVELOPE FLAP

animal	location	pre	post-surgery	%	post-suture	%
572	max	.11	.50	454	.11	100
572	man	.24	.05	20	.08	33
992	max	.25	.14	56	.11	44
992	man	.20	.15	75	.17	85
110	max	.09	.09	100	.08	88
110	man	.08	.03	37	.04	50
mean		.16167	.16000	133.6	.09833	66.6
SD		.07731	.17321	164.2	.04355	27.6
Tail probability		.5427				
Greenhouse-Geisser		.4716				
Huyhn-Felt		.4826				
adjusted mean				57		66.6
SD				31.4		27.6

MIDDLE ENVELOPE FLAP

animal	location	pre	post-surgery	%	post-suture	%
572	max	1.02	.51	50	.41	40
572	man	.12	.15	125	.12	100
992	max	.52	.18	34	.14	26
992	man	.24	.24	100	.24	100
110	max	.31	.34	109	.38	122
110	man	.10	.10	100	.14	140
mean		.38500	.25333	86	.23833	69.6
SD		.34628	.15042	35	.12875	45.4
Tail probability		.2252				
Greenhouse-Geisser		.2466				
Huyhn-Felt		.2465				
adjusted mean				86		69.6
SD				35		45.4

BASE ENVELOPE FLAP

animal	location	pre	post-surgery	%	post-suture	%
572	max	.91	.55	60	.90	98
572	man	.35	.14	40	.17	48
992	max	.42	.73	173	.76	180
992	man	.36	.63	175	.42	130
110	max	.23	.34	147	.33	143
110	man	.91	.30	32	.40	43
mean		.53000	.44833	104.5	.49666	107
SD		.30073	.22427	67.6	.27631	54.4
Tail probability		.7913				
Greenhouse-Geisser		.7053				
Huyhn-Felt		.7476				
adjusted mean				104.5		107
SD				67.6		54.4

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